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Intraspecific Variations and Postglacial Distribution of Lake Char (*Salvelinus namaycush*)

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KHAN, N. Y., AND S. U. QADRI. 1971. Intraspecific variations and postglacial distribution of lake char (*Salvelinus namaycush*). J. Fish. Res. Bd. Canada 28: 465–476.

A morphometric and meristic study of 325 lake char (*Salvelinus namaycush*) from over its range of distribution showed different gene pools. The similarities and differences observed among lake char of various primary ocean drainages and among various populations of lake char within these drainages suggested that the species survived the last glaciation in unglaciated Alaska and Yukon, possibly other unglaciated northern areas, and at least three refugia in the south, the Atlantic, Upper Mississippi, and Upper Missouri.

Received July 2, 1970

IN this study an attempt has been made to determine the extent of intraspecific morphological variations over the distributional range of lake char[1] (*Salvelinus namaycush*) and to explain its glacial refugia and postglacial dispersal routes.

Lake char is distributed all over northern North America, from the islands of the Canadian Arctic Archipelago south to the upper Mississippi valley, in the Great Lakes basin, and from British Columbia and Alaska east to Labrador. It has not been reported from the island of Newfoundland. West of the continental divide it is present in the upper Fraser River but absent from the Columbia River. It is also absent from the Pacific drainages north of Cook Inlet (Lindsey 1964).

Conflicting theories have been put forth to explain its glacial refugia and postglacial dispersal routes. Radforth (1944) believed that the lake char survived the Wisconsin glaciation in the unglaciated parts of Alaska, whereas Walters (1955) and McPhail (MS 1963) advocated a southern refugium in the Mississippi valley. Wynne-Edwards (1947, 1952) also believed in a southern refugium with the possibility of a northern refugium in the unglaciated parts of the Yukon River basin. Lindsey (1964) believed in multiple southern and northern refugia for the lake char.

Materials and Methods

A total of 325 specimens of lake char, representing 65 localities (Fig. 1 and Table 1) from throughout its range, were examined for 12 morphometric and three

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[1] Although the generally accepted name for this species is "lake trout," the authors prefer to retain the name "lake char" as used in Qadri (1967). Also, Qadri maintains *Cristovomer* as the generic name.

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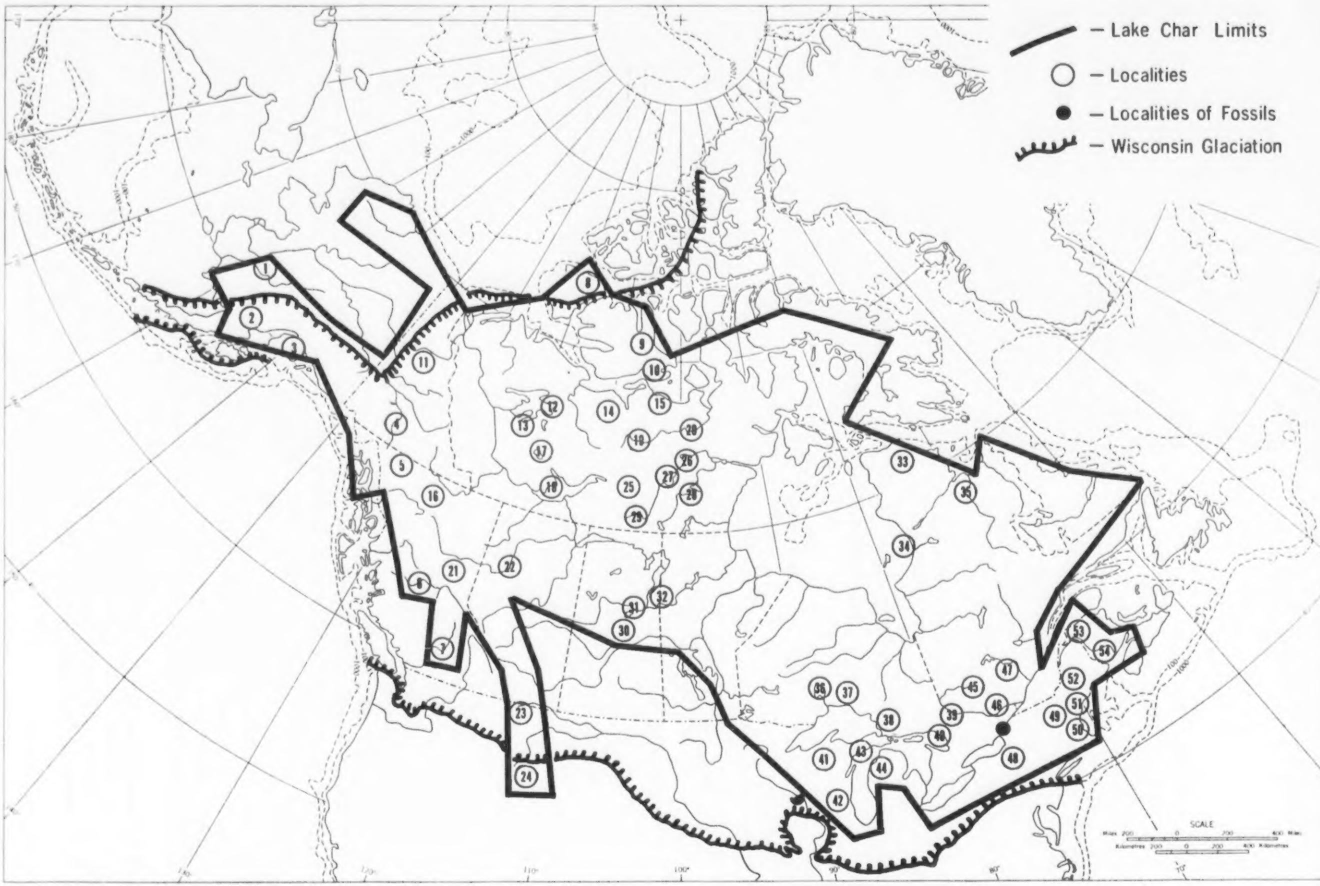

– Lake Char Limits
○ – Localities
● – Localities of Fossils
– Wisconsin Glaciation
SCALE
Miles 200 0 200 400 Miles
Kilometres 200 0 200 400 Kilometres
120°
110°
100°
90°
80°
70°

FIG. 1. Limits of endemic lake char distribution based on the localities considered and Lindsey (1964). The glacial limits show only the last or Wisconsin glaciation. Solid circles show the localities of the lake char fossils. For the description of localities see Table 1.

TABLE 1. Localities of lake char samples studied, numbers, and lengths.

Drainage	Area no.	Locality no.	Locality	Location		Fork length[a] (*mm*)	
				N	W	Areas	Drainages
Pacific							237.0–481.5
							(376.8)16
	1	1	Aniak L., Alaska	62°00′	160°00′	237.0–443.0	
		2	Kokhanok L., Alaska	59 24	154 10	(348.2)9	
		3	Upper Trail L., Alaska	61 00	149 00		
	2	4	L. Laberge, Yukon	61 10	135 35	333.0–480.0	
		5	Atlin L., B.C.	59 00	133 00	(432.7)4	
	3	6	Cunningham L., B.C.	54 00	124 00	273.0–481.5	
		7	Canim L., B.C.	51 00	120 00	(388.1)3	
Arctic							83.0–650.0
							(353.7)124
	4	8	Banks Is., N.W.T.	74 03	119 43	249.0–590.0	
		9	Zeta L., N.W.T.	71 00	106 38	(464.3)16	
		10	Ferguson L. and	69 28	104 25		
			Greiner L., N.W.T.	69 07	105 10		
	5	11	Fairchild L., Yukon	64 58	133 43	83.0–180.0	
						(137.9)10	
	6	12	Great Bear L. and	66 14	117 50	162.0–588.0	
			Spark Plug L., N.W.T.	66 07	117 52	(377.3)27	
		13	Keller L., N.W.T.	63 55	121 35		
	7	14	Kathawachaga L., N.W.T.	66 14	110 43	276.0–490.0	
		15	Ellice L., N.W.T.	68 02	103 28	(370.1)16	
	8	16	Simmons L., B.C.	59 00	129 00	252.0–348.0	
						(310.5)6	
	9	17	Lac la Martre, N.W.T.	63 08	116 54	165.0–650.0	
		18	Great Slave L., N.W.T.	61 23	115 38	(388.7)17	
	10	19	Beechy L., N.W.T.	65 12	106 28	271.0–525.0	
		20	MacDougal L., N.W.T.	65 58	98 37	(376.4)15	
	11	21	Tachaeda L., B.C.	54 15	122 30	168.0–475.0	
		22	Swan L., Alta.	55 04	117 49	(221.4)17	
Mexico							
	12	23	St. Mary's Glacier, Mont.	48 42	113 30	221.0–431.0	
		24	Elk L., Mont., and	44 41	111 37	(349.8)6	
			Heart L., Wyo.	44 25	110 50		
Hudson							176.0–657.0
							(410.3)65
	13	25	Whitefish L., N.W.T.	62 36	106 43	176.0–569.0	
		26	Beverly L. and	64 36	100 38	(338.4)35	
			Aberdeen L., N.W.T.	64 27	99 00		
		27	Dubawont L., N.W.T.	63 08	101 13		
		28	Angikuni L., N.W.T.	62 13	99 50		
		29	Wholdaia L., N.W.T.	60 43	104 10		
	14	30	L. Waskesieu, Sask.	53 57	106 15	410.0–580.0	
		31	Lac la Ronge, Sask.	55 00	105 00	(504.9)21	
		32	Mackay L., Sask.	55 27	104 56		
	15	33	Museum L., Que.	61 17	73 40	372.0–657.0	
		34	Lakes on Broughton Is., Que.	57 23	76 48	(468.4)9	
		35	Koksoak R., Que.	58 00	68 00		
Atlantic							169.0–679.0
							(366.1)114
	16	36	L. Nipigon, Ont.	50 00	88 50	295.0–571.0	
		37	Long L., Ont.	49 50	87 00	(416.8)18	
		38	Rock L., Ont.	47 00	83 50		

(*Continued*)

TABLE 1. Localities of lake char samples studied, numbers, and lengths. (*Concluded*)

Drainage	Area no.	Locality no.	Locality	Location		Fork length[a] (*mm*)	
				N	W	Areas	Drainages
Atlantic (*con't.*)		39	L. Timagami, Ont.	47°00′	80°05′		
		40	Cache L. and	47 50	91 00		
			Ragged L., Ont.	46 15	78 45		
	17	41	Trout L., Wis.	46 05	89 40	169.0–545.0	
		42	Crystal L., Wis.	43 45	88 00	(347.2)27	
		43	Pine L.,	46 53	87 52		
			Mountain L.,	46 51	87 55		
			Rush L., and	46 54	87 55		
			First Pine L., Mich.	46 51	87 51		
		44	Elk L. and	45 50	85 25		
			Torch L., Mich.	45 00	85 00		
	18	45	Lac aux Sangsues, Que.	46 30	77 57	218.0–586.0	
		46	Dodds L., Que.	45 42	75 36	(343.0)54	
		47	Saccacomie L. and	46 29	73 14		
			Normand L., Que.	46 29	73 14		
	19	48	Owasco L., N.Y.	43 00	76 50	211.0–679.0	
		49	Stinson L., N.H.	43 50	71 45	(421.0)15	
		50	Winnisquam L. and	43 30	71 30		
			Squam L., N.H.	43 45	71 31		
		51	L. Winnipesaukee, N.H.	43 30	71 20		
		52	Moosehead L., Maine	45 45	69 45		
		53	Baker L., N.B.	47 21	68 41		
		54	Chamkook L., N.B.	45 10	67 05		

[a] Range of fork lengths, mean in parentheses, and number of fish after the parentheses.

meristic characters. Lake char were not easy to obtain, and although it would have been advantageous to examine more material, additional specimens were not available. Since some localities were too close to each other to be shown distinctly on the map, they are indicated by a single number; e.g., under locality no. 10 are included Ferguson and Greiner lakes.

MORPHOMETRIC CHARACTERS

The measurements were taken on the left side of the fish with the help of a divider and follow those of Hubbs and Lagler (1958), Ouellette and Qadri (1966), and Qadri (1967): fork length, predorsal length, ventral–anal length, length of caudal peduncle, dorsal-adipose length, depth of caudal peduncle, body depth, head length, interorbital width, postorbital length of head, length of upper jaw, and length of lower jaw.

MERISTIC CHARACTERS

Alizarin red S staining (Evans 1948) and radiography were used to study the following meristic characters:

Number of vertebrae — In all fish, the last upturned caudal vertebrae were counted as three. Specimens with fused vertebrae were not used for vertebral counts.

Interneurals — All single elements anterior to the dorsal fin pterigiophores were counted. The first interneural that was flat and platelike was included in the counts.

Number of gill rakers — The first left gill arch was removed from each specimen and all the gill rakers, developed and undeveloped, were counted under a stereomicroscope.

As meristic characters were hard to determine in some specimens, the number of fish examined in some samples was small.

STATISTICAL PROCEDURES

For the study of the morphometric characters, the relative growth method developed by Huxley (1932) was used. The computations for regression analysis were done after logarithmic transformation of the data and the significance of regression coefficients was tested by a t-test at $P = 0.05$ (Steel and Torrie 1960). For each meristic character a one-way analysis of variance was performed and the significance of difference among the means was tested by the new Duncan's multiple range test (Duncan 1955) with Kramer's modification (Kramer 1956) at $P = 0.05$.

For comparison of samples, the 65 localities were grouped in two fashions (Fig. 2). In the first instance, the localities within each primary ocean drainage (Pacific, Arctic, Hudson, and Atlantic) were lumped to see the interdrainage variations in lake char populations.

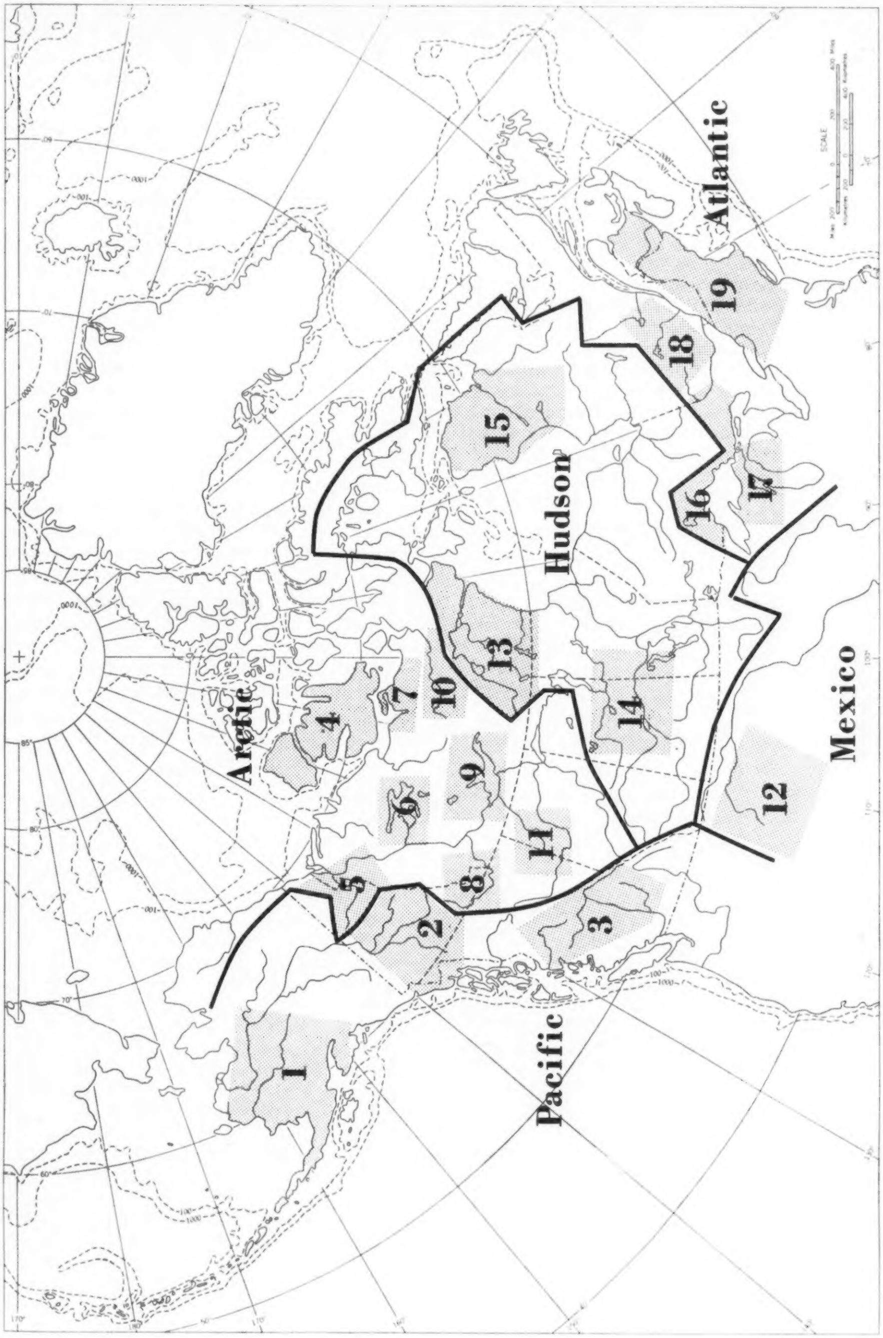

Arctic
Pacific
Hudson
Atlantic
Mexico
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
SCALE

FIG. 2. The five primary ocean drainages in northern North America and 19 areas from which lake char were compared.

Secondly, within each primary drainage the localities were grouped into areas. The 19 areas so demarcated over the range of the species represent either a secondary watershed or an area of geographical and geological importance. The sample from the Gulf of Mexico drainage was not included in morphometric studies because of its small size.

To interpret the morphometric variations among the lake char of various areas, only four characters (head length, lengths of upper and lower jaws, and predorsal length) were considered. Since all the areas did not have a sufficiently large sample size for the morphometric comparisons, only those areas that were represented by 15 or more specimens were selected for comparisons. These areas were 4, 6, and 11 in the Arctic drainage; 13 and 14 in the Hudson drainage; 17, 18, and 19 in the Atlantic drainage. The lake char of areas 1, 2, and 3 in the Pacific drainage did not show any significant difference from each other in morphometric characters and therefore were treated as one sample. The significant differences in the morphometric characters among areas were ranked as: none, one, two, three, and four, to determine their relationship.

Results and Discussion

MORPHOMETRIC CHARACTERS

The combined samples of lake char from each of the primary ocean drainages showed that the Pacific drainage fish did not differ from either the Arctic or the Atlantic drainage fish but differed from the Hudson drainage fish in the predorsal length and the length of caudal peduncle (Table 2). The Arctic drainage lake char differed from the Hudson and Atlantic drainage fish in four and seven characters, respectively. The Hudson drainage fish differed from those of the Atlantic in six characters. These interdrainage comparisons of morphometric characters suggest that the lake char populations in these drainages may have developed different gene pools.

The interarea comparisons (Fig. 3; for tables of regression coefficients and significance of regression coefficients see Khan MS 1968) of morphometric characters showed that the Pacific drainage lake char differed only from area 11 fish in two characters.

Within the Arctic drainage, area 11 lake char differed from those of areas 6 and 4 in three characters, whereas those of areas 6, 4, and 10 did not differ from each other. Area 11 fish also differed from those of areas 13 and 14 (Hudson drainage), and those of areas 17, 18, and 19 (Atlantic drainage) in four, three, three, four, and three characters, respectively. This suggests that the area 11 lake char (Peace River system) did not originate from either the Atlantic or Mississippi refugium. The unglaciated parts of Montana could have served as refugia for area 11 lake char. Natural populations of lake char still occur in the upper Missouri River system (Henshall 1907; Schultz 1941; Vincent 1963). Lindsey (1964) gave two explanations for the occurrence of natural populations of lake char in Montana. Firstly, the lakes in Montana with lake char populations lie immediately south of the Saskatchewan River basin and could have received lake char from the Yukon River system

TABLE 2. Significance of regression coefficients of head and body parts on fork lengths of the lake char from Pacific, Arctic, Hudson, and Atlantic drainages.

	Pacific vs. Arctic		Pacific vs. Hudson		Pacific vs. Atlantic		Arctic vs. Hudson		Arctic vs. Atlantic		Hudson vs. Atlantic	
Character	df[a]	*t*[b]	df	*t*	df	*t*	df	*t*	df	*t*	df	*t*
Head length	134	0.867	68	1.787	122	–0.018	174	2.461*	228	–2.678*	162	–3.948*
Postorbital length	133	0.714	67	0.754	121	–0.129	172	0.905	226	–2.031*	160	–1.787
Interorbital width	133	0.944	68	0.270	121	–0.018	173	–2.016*	226	–3.172*	161	–0.668
Length of upper jaw	130	0.563	67	1.479	118	–0.160	169	1.816	220	–2.355*	157	–3.900*
Length of lower jaw	138	0.829	67	1.789	121	0.078	169	2.110*	223	–2.410*	160	–3.796*
Predorsal length	133	0.976	68	2.502*	125	0.346	173	4.359*	230	–1.109	165	–3.497*
Ventral-anal length	134	0.778	68	0.480	124	1.316	174	–1.151	230	1.391	164	2.287*
Length of caudal peduncle	132	–1.442	68	–2.012*	126	–0.468	172	–1.127	230	3.223*	166	3.561*
Dorsal-adipose length	134	–0.570	68	–0.812	124	–0.239	174	–0.194	230	1.002	164	0.974
Depth of caudal peduncle	134	–1.261	67	1.176	125	1.354	173	–0.948	231	–0.102	164	0.835
Body depth	131	0.876	68	0.430	119	0.250	171	–1.632	222	–2.512*	159	–0.551

[a] Degrees of freedom.

[b] Student's *t*-test values; *significant at $P = 0.05$.

through a temporary ice-free corridor between the Cordilleran and Keewatin ice sheets. Secondly, he suggested that the populations of lake char existed throughout the last glaciation in Montana and moved north with the retreating ice during deglaciation. The latter hypothesis is more plausible and is supported by our results. The lake char of area 11 differed from the Pacific drainage lake char. The lake char of the Peace River system, therefore, probably originated from the upper Missouri refugium.

The lake char of areas 13 and 14 (Hudson drainage) did not differ from each other and presumably have a fairly homogeneous gene pool. Fish from

TABLE 3. Regressions of lengths of head and body parts on fork lengths of lake char from Pacific (Pac), Arctic (Arc), Hudson (Hud), and Atlantic (Atl) drainages.

Character	Ocean drainage	Fork length (*mm*)	Regression coefficient	Constant
Head length	Pac	237.0–481.0	1.093±0.061[a]	–0.851
	Arc	83.0–650.0	1.039±0.010	–0.712
	Hud	176.0–580.0	0.985±0.018	–0.572
	Atl	169.0–679.0	1.094±0.019	–0.868
Interorbital width	Pac	237.0–481.0	1.133±0.056	–1.493
	Arc	83.0–650.0	1.051±0.015	–1.291
	Hud	176.0–580.0	1.113±0.024	–1.460
	Atl	169.0–679.0	1.135±0.021	–1.504
Postorbital length	Pac	237.0–481.0	1.130±0.061	–1.183
	Arc	83.0–650.0	1.087±0.010	–1.064
	Hud	176.0–580.0	1.064±0.029	–1.001
	Atl	169.0–679.0	1.145±0.031	–1.224
Length of upper jaw	Pac	237.0–481.0	1.189±0.077	–1.372
	Arc	83.0–650.0	1.131±0.017	–1.232
	Hud	176.0–580.0	1.066±0.026	–1.060
	Atl	169.0–679.0	1.203±0.023	–1.426
Length of lower jaw	Pac	237.0–481.0	1.174±0.070	–1.249
	Arc	83.0–650.0	1.107±0.013	–1.083
	Hud	176.0–580.0	1.048±0.021	–0.931
	Atl	169.0–679.0	1.168±0.021	–1.251
Predorsal length	Pac	237.0–481.0	1.098±0.030	–0.581
	Arc	83.0–650.0	1.065±0.006	–0.486
	Hud	176.0–580.0	1.011±0.011	–0.354
	Atl	169.0–679.0	1.080±0.014	–0.530
Ventral-anal length	Pac	237.0–481.0	1.025±0.068	–0.798
	Arc	83.0–650.0	0.960±0.014	–0.619
	Hud	176.0–580.0	0.993±0.020	–0.690
	Atl	169.0–679.0	0.926±0.019	–0.528
Length of caudal peduncle	Pac	237.0–481.0	0.875±0.060	–0.510
	Arc	83.0–650.0	0.962±0.010	–0.745
	Hud	176.0–580.0	0.986±0.016	–0.796
	Atl	169.0–679.0	0.903±0.015	–0.597
Dorsal-adipose length	Pac	237.0–481.0	0.933±0.055	–0.535
	Arc	83.0–650.0	0.975±0.012	–0.643
	Hud	176.0–580.0	0.980±0.017	–0.659
	Atl	169.0–679.0	0.952±0.020	–0.566
Depth of caudal peduncle	Pac	237.0–481.0	1.033±0.073	–1.212
	Arc	83.0–650.0	0.934±0.013	–0.960
	Hud	176.0–580.0	0.960±0.018	–1.020
	Atl	169.0–679.0	0.936±0.018	–0.978
Body depth	Pac	237.0–481.0	1.131±0.144	–1.037
	Arc	83.0–650.0	1.023±0.019	–0.767
	Hud	176.0–580.0	1.088±0.024	–0.938
	Atl	169.0–679.0	1.107±0.023	–0.974

[a] One standard error of coefficient.

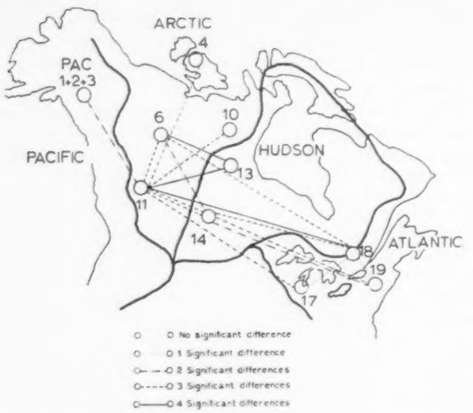

ARCTIC
4
PAC
1+2+3
6
10
HUDSON
PACIFIC
13
11
14
ATLANTIC
18
19
17
O No significant difference
O 1 Significant difference
O 2 Significant differences
O 3 Significant differences
O 4 Significant differences

FIG. 3. Ranked morphometric differences among lake char of Pacific drainage (1+2+3) and areas, 4, 6, 10, 11, 13, 14, 17, 18, and 19. Areas not joined by any line do not show significant differences.

areas 13 and 14 differed from those of areas 6 and 11 (Arctic drainage). Fish from area 14 differed from those of areas 18 and 19 (Atlantic drainage).

Within the Atlantic drainage area 18 lake char differed from those of areas 17 and 19 in only one character. The area 17 lake char did not differ from those of areas 13 and 14 (Hudson drainage) nor from those of areas 4, 10, and 6 (Arctic drainage). Area 17 lake char are from lakes close to the Mississippi drainage; therefore, it is logical to say that the lake char originating from the upper Mississippi refugium dispersed north into the present Hudson drainage and the northern Arctic drainages. There is enough paleontological evidence to support this conclusion. A fossil lake char from Wisconsin close to the southern edge of glaciation within the present Mississippi drainage (Fig. 1) was assigned by Hussakof (1916) to the interglacial period between the second and third Pleistocene glaciations. The recent radiocarbon dating techniques assign this fossil to the closing stages of the last or Wisconsin glaciation, i.e., 12,500–16,000 B.P. (before present) (Lindsey 1964). Recently another fossil lake char has been described from the Ottawa region (Gruchy 1968). Its estimated age is 9500 ± 300 years, suggesting that lake char closely followed the northward retreat of the ice sheet.

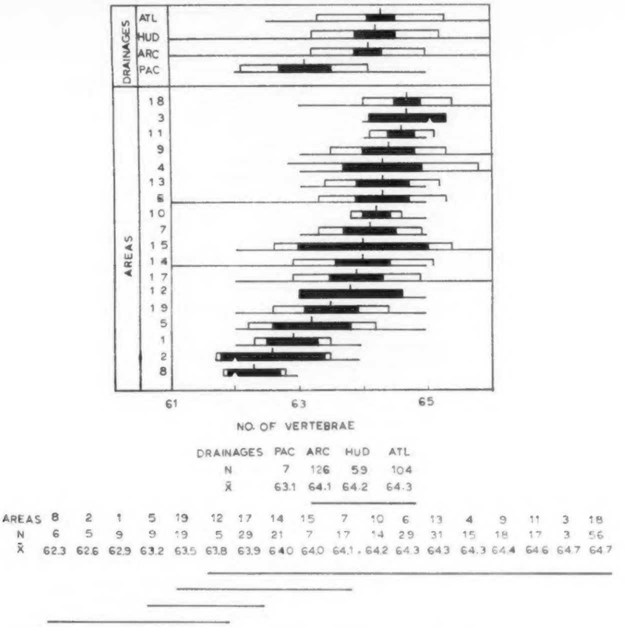
Radforth (1944) argued against a Mississippi refugium south of the ice sheet. Her objection was based upon the absence of suitable habitats for lake char in that area. However, in Wisconsin there are still natural lake char populations close to the southern edge of glaciation. This, according to Lindsey (1964), does not "constitute proof of a Mississippi refugium," since some divides between the Great Lakes and Mississippi watershed are swampy, which would be too warm a route to be followed by an essentially cold-water fish like lake char. However, the topographical conditions of an area are not static and these conditions may not have existed during the early stages of deglaciation.

Also the lake char originating from the Mississippi refugium apparently shared gene pools with those originating from the Atlantic refugium. This is suggested by the absence of morphometric differences between areas 17 and 19 lake char. The area 18 populations differed from those of areas 19 and 17. This differentiation in morphometric characters probably occurred due to the isolation of lake char populations on either side of the St. Lawrence lowlands during the Champlain Sea episode. Deglaciation on the Atlantic coast occurred earlier and more rapidly than in the upper Mississippi area and the Great Lakes basin. The initial ice retreat in the Long Island Sound area began about 30,000 B.P. (Newman and Fairbridge 1960) and most of Nova Scotia became ice-free by 10,000 B.P. (Hickox 1960). It is suggested that the lake char survived the glaciation in a glacial refuge on the Atlantic coast (McPhail MS 1963), and invaded southeast (area 19) as well as northwest (area 18) of the St. Lawrence lowlands. The deglaciation on the Atlantic coast was followed by marine submergence (Flint 1957) and the sea flooded the St. Lawrence lowlands forming the Champlain Sea. The Champlain Sea episode occurred about 10,000–12,000 B.P. and lasted for about 1000–2000 years (Terasmae 1959) and probably isolated populations of lake char on both sides of St. Lawrence lowlands.

MERISTIC CHARACTERS

The number of vertebrae and interneurals presented similar patterns of variation. The mean values for both these characters (Fig. 4 and 5) were significantly lower in the Pacific drainage lake char than in those of the Arctic, Hudson, and Atlantic drainages. No significant variations were observed in these characters among the Arctic, Hudson, and Atlantic lake char.

The mean number of vertebrae in the lake char of areas 1 and 2 of the Pacific drainage were not different from each other, nor from those of areas 5 and 8 in the Arctic drainage and area 19 in the Atlantic drainage. Area 3 lake char in the Pacific drainage differed significantly from those of other areas in the same drainage (Fig. 4). Similarly, the



DRAINAGES	PAC	ARC	HUD	ATL
N	7	126	59	104
X̄	63.1	64.1	64.2	64.3

AREAS	8	2	1	5	19	12	17	14	15	7	10	6	13	4	9	11	3	18
N	6	5	9	9	19	5	29	21	7	17	14	29	31	15	18	17	3	56
X̄	62.3	62.6	62.9	63.2	63.5	63.8	63.9	64.0	64.0	64.1	64.2	64.3	64.3	64.3	64.4	64.6	64.7	64.7

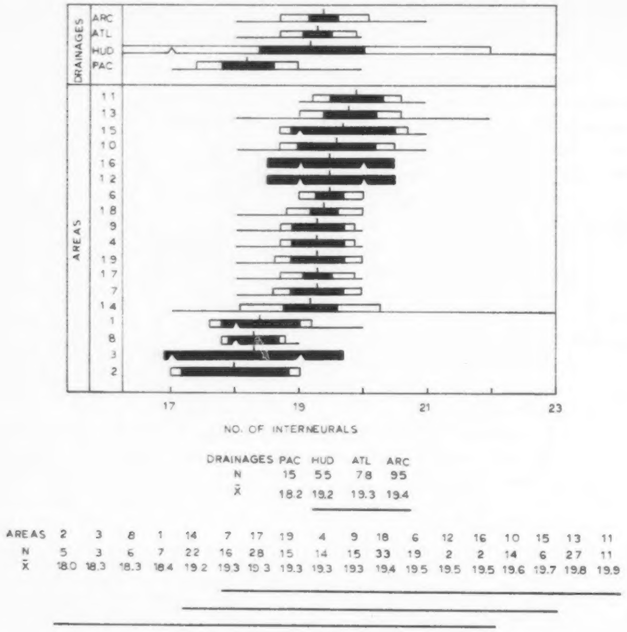
FIG. 4. Variation in vertebral counts in the lake char from the four primary ocean drainages and from the areas in each drainage. Horizontal lines, ranges; vertical lines on bars, means; solid bars, four standard errors of the means; and open and solid bars together, two standard deviations of the means. Below, any two means underscored by the same line are not different ($P = 0.05$) by Duncan's test.

mean numbers of interneurals in the lake char of areas 1, 2, and 3 did not differ among each other, nor from area 8 in the Arctic drainage (Fig. 5).

For the lake char, Vladykov (1954) mentioned a mean of 63.0 vertebrae; Norden (1961) 63.9; Qadri (MS 1964) 64.2; and Slastenenko (1958) a range of 64–65 vertebrae. These values fall within the range of variations observed in this study.

The variations in these characters support the hypothesis that lake char survived the glaciation north as well as south of the ice sheet. The Pacific drainage populations of lake char (areas 1, 2, and 3) and the Arctic drainage populations (areas 5, 8, and 11) show relatively lower mean values for these characters than those in the other areas. Areas 5, 8, and 11 are in the Peel, Liard, and Peace River systems, respectively; these rivers are in the Arctic drainage, yet they extend deep into Yukon and Fraser valleys, which are in the Pacific drainage. It appears, therefore, that at some time since glaciation these rivers have shared headwaters and that there has been some gene flow in lake char populations from the Yukon River basin to the Peel (area 5) and Liard (area 8) River systems; and from the Peace River system (area 11) to the Fraser River system (area 3). Wynne-Edwards (1947) had suggested the Peel and Liard rivers as the possible dispersal routes for the lake char.

The mean number of gill rakers (21.5) in the Atlantic drainage lake char differed significantly from those of Hudson (22.7), Arctic (23.2), and Pacific (22.9) drainage fish (Fig. 6). Among lake char of various areas, the mean values of the total number of gill rakers did not show the trend of variation seen in metameric characters, i.e., the vertebrae and interneurals. Vladykov (1954) mentioned a mean of 20.2 gill rakers, and Qadri (MS 1964) 22.4. Martin and Sandercock (1967) gave mean values for the total number of gill rakers for the lake char of three Algonquin Park lakes: Lake Opeongo (21.06), Happy Isle (22.05), and Lake Louisa (22.26). All these values agree quite closely with those of our samples drawn from similar areas.



DRAINAGES	PAC	HUD	ATL	ARC
N	15	55	78	95
x̄	18.2	19.2	19.3	19.4

AREAS	2	3	8	1	14	7	17	19	4	9	18	6	12	16	10	15	13	11
N	5	3	6	7	22	16	28	15	14	15	33	19	2	2	14	6	27	11
x̄	18.0	18.3	18.3	18.4	19.2	19.3	19.3	19.3	19.3	19.3	19.4	19.5	19.5	19.5	19.6	19.7	19.8	19.9

FIG. 5. Variation in interneural counts in the lake char from the four primary ocean drainages and from the areas in each drainage. Horizontal lines, ranges; vertical lines on bars, means; solid bars, four standard errors of the means; and open and solid bars together, two standard deviations of the means. Below, any two means underscored by the same line are not different ($P = 0.05$) by Duncan's test.

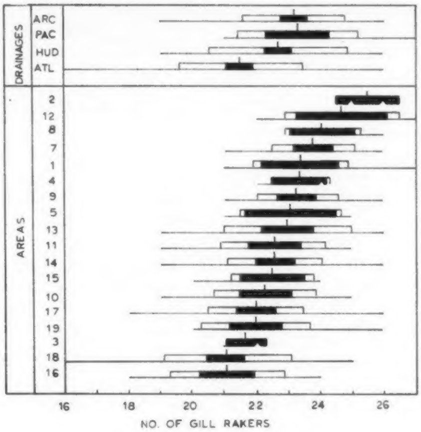
Among lake char of various areas, the mean values of gill rakers did not show any definite trend of variations.

The above results clearly point out the fact that the lake char does not form a homogeneous gene pool over its distributional range. However, no latitudinal clines were noticed. This could be explained on the basis of homogeneous environment of those lakes that the lake char inhabit. Latitudinal clines are the result of variable temperatures, which the lake char does not experience. Therefore, the variations observed in lake char at present are mainly due to long isolation of populations during the last or Wisconsin glaciation. Most geneticists agree that mutations in fish are induced by naturally occurring radiation. Rounsefell (1962) pointed out that lake char inhabit deeper waters and receive much less total radiation than salmonids inhabiting surface waters and spending some time in streams. This lowered mutation rate has slowed down the evolutionary process in lake char. However, lake char are possibly subject to some radiation, in the egg stage or during extended periods spent on the bottom. Probably, this is how the homogeneity of the gene pool is broken down. The subspecific distinction of populations is unlikely, since there is a great deal of overlap in variations. Local ecological subspeciation has been reported in the Lake Superior populations of lake char (Khan and Qadri 1970). Such ecological subspeciation could be present elsewhere over the range of the species but it was impossible to determine on the basis of the above data.

Conclusions

Lake char from the Peace River system differ morphometrically from those of Pacific, Arctic, Hudson, and Atlantic drainages, suggesting their origin from a refugium in the unglaciated parts of Montana.

The Hudson drainage populations of lake char form a fairly homogeneous gene pool. They do not differ from those populations of lake char in the



DRAINAGES	ATL	HUD	PAC	ARC
N	125	51	14	80
x̄	21.5	22.7	22.9	23.3

AREAS	16	18	3	19	17	10	15	14	11	13	5	9	4	1	7	8	12	2
N	16	58	3	19	32	15	8	21	16	22	6	15	5	9	17	6	6	2
x̄	21.1	21.1	21.7	22.0	22.0	22.3	22.5	22.6	22.6	23.0	23.2	23.3	23.4	23.4	23.8	24.2	24.7	25.5

FIG. 6. Variation in gill-raker counts in the lake char from the four primary ocean drainages and from the areas in each drainage. Horizontal lines, ranges; vertical lines on bars, means; solid bars, four standard errors of the means; and open and solid bars together, two standard deviations of the means. Below, any two means underscored by the same line are not different ($P = 0.05$) by Duncan's test.

Atlantic drainage that are close to the Mississippi drainage. This suggests that they originated from a Mississippi refugium.

Lake char also survived in the Atlantic refugium. During deglaciation, populations got isolated on either side of the St. Lawrence lowlands and also shared gene pools with those surviving in the Mississippi drainage.

Study of meristic characters suggests that lake char also survived glaciation in Alaska and the Yukon and shared gene pools with those originating from the south through Peel and Liard river drainages.

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Histological Effects of 11-Ketotestosterone, 17α-Methyltestosterone, Estradiol, Estradiol Cypionate, and Cortisol on the Interrenal Tissue, Thyroid Gland, and Pituitary Gland of Gonadectomized Sockeye Salmon (*Oncorhynchus nerka*)

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VAN OVERBEEKE, A. P., AND J. R. MCBRIDE. 1971. Histological effects of 11-ketotestosterone, 17α-methyltestosterone, estradiol, estradiol cypionate, and cortisol on the interrenal tissue, thyroid gland, and pituitary gland of gonadectomized sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 28: 477–484.

Effects of two androgens, two estrogens, and cortisol in gonadectomized sockeye salmon (*Oncorhynchus nerka*) were investigated. Males were injected with 11-ketotestosterone, 17α-methyltestosterone, or cortisol for 4 or 7 weeks. Females were injected with estradiol, estradiol cypionate, or cortisol for 8 weeks.

Both the androgens and the estrogens caused interrenal hypertrophy. The effect of 17α-methyltestosterone was stronger than that of 11-ketotestosterone, and of the two estrogens used, estradiol cypionate exerted the most marked effect. Cortisol brought about a slight atrophy of the interrenal tissue.

A pronounced histological activation of the small thyroid follicles was induced by the two androgens. The estrogens exerted no visible effect on the thyroid gland, and cortisol treatment caused a slight reduction in the height of the follicular epithelium.

In the gonadectomized control fish of both sexes, gonadotrophs were small and practically all devoid of granules. All four sex steroids induced the appearance of many granulated PAS-positive gonadotrophs, particularly in the anterior part of the proximal pars distalis. This effect is considered on activation of the gonadotrophs. Cortisol administration induced pronounced degranulation and atrophy of the corticotrophs and this effect was strongest in the males. Neither the androgens nor the estrogens visibly affected the corticotrophs.

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GONADECTOMY of sockeye salmon (*Oncorhynchus nerka*) performed during the last stages of sexual maturation prolongs the life span of these fish well beyond the time at which they normally would have reproduced and died (McBride et al. 1963; McBride and van Overbeeke 1969a, b). This operation has been shown to interfere with the development and maintenance of secondary sexual characteristics and to affect the pituitary gland, in particular the condition of the gonadotrophic cells (McBride and van Overbeeke 1969a).

In addition, gonadectomy leads to a cessation or partial reversal of the alterations in various other tissues normally occurring in sexually maturing and spawning salmon. Specifically, it was shown to prevent or reverse interrenal hypertrophy (McBride and van Overbeeke 1969b) and to prevent the increase in volume of distribution and metabolic clearance rate of cortisol (Donaldson and Fagerlund 1968a, b).

This last result is of particular significance, since hyperadrenocorticism has been considered a direct cause of the deteriorative tissue changes observed in Pacific salmon during the final stages of migration and reproduction (Hane and Robertson 1959; Robertson and Wexler 1959, 1960, 1961; Robertson et al. 1961a, b, 1963).

To further analyse the role of the gonads in the development of hyperadrenocorticism, investigations were undertaken to study the effects of gonadal hormones on several endocrine organs as well as on the development of degenerative tissue changes in gonadectomized sockeye salmon of both sexes. The effects were compared with those of the corticosteroid cortisol. The results concerning the effects on some physiological parameters of the adrenocortical activity have been published previously (Donaldson and Fagerlund 1969; Fagerlund and Donaldson 1969). This report concerns the histological effect of 11-ketotestosterone, 17α-methyltestosterone, estradiol, estradiol

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cypionate, and cortisol on the interrenal tissue, thyroid glands, and pituitary gland.

Materials and Methods

FISH

Sexually immature sockeye salmon were caught in June 1967 when they entered Great Central Lake on Vancouver Island, B.C., during their migration to the spawning grounds. The methods of capture and transport to the Vancouver laboratory have been described earlier (McBride et al. 1963). Within 2–3 weeks after their arrival in the laboratory, they were gonadectomized (McBride et al. 1963) and 3–4 weeks later the fish were induced to feed on a preparation of marine zooplankton (McBride 1967). Throughout the experiment, the fish were maintained under a natural photoperiod. The temperature of the water in the holding tanks ranged from a high of 14 C (in September) to a low of 4 C (in February). A total of 76 fish (42 males and 34 females) was used. The mean weight of the males was 1947 g and of the females 1682 g. These fish were selected from a larger group of gonadectomized sockeye on the basis of complete absence of nuptial coloration and secondary sexual characteristics, which indicated that removal of gonadal tissue had been complete. This last indication was confirmed at autopsy.

EXPERIMENTAL PROCEDURE

Males

Intramuscular hormone injections commenced in October, 3 months after gonadectomy, and were given twice weekly for a period of 7 weeks. There were 4 groups: group 1 were controls, of which six were killed at the beginning of the experiment, four after 4 weeks, and six when the experiment was terminated; group 2 received 11-ketotestosterone; four of these were killed after 4 weeks and four after 7 weeks; group 3 was injected with 17α-methyltestosterone and samples of five fish each were killed after 4 and 7 weeks; group 4 were cortisol-injected fish, six of which were killed after 4 weeks and two after 7 weeks. All hormones were injected in dosages of 2.50 mg dissolved in 0.2 ml of peanut oil. Control fish were given 0.2 ml of peanut oil twice weekly.

Females

Hormone injections were given intramuscularly for a period of 8 weeks beginning in January, 6–7 months after gonadectomy. There were 4 groups: group 5 served as controls and received 0.2 ml of peanut oil twice weekly. Five controls were killed at the beginning of the experiment and seven after 8 weeks; group 6 (eight fish) received 2.50 mg estradiol in 0.2 ml of peanut oil twice per week; group 7 (eight fish) was injected with 1.0 mg estradiol cypionate (Depo-Estradiol, The Upjohn Co., Don Mills, Ont.) in cotton seed oil, once per week; group 8 (six fish) received 2.50 mg cortisol in 0.2 ml of peanut oil once per week. Treatment was continued for 8 weeks, after which the fish were killed. All injections were made after the fish were anaesthetized in 2-phenoxyethanol.

HISTOLOGICAL TECHNIQUES

Fish were killed by severing the spinal cord, just caudal to the head. Samples of the headkidneys, containing the interrenal tissue, parts of the subpharyngeal area, containing the thyroid follicles, and the pituitary glands were dissected out and fixed in Bouin–Hollande–Sublimate fixative, processed, and embedded in Paraplast, mp 56 C. Series of 5-μ thick sections, either transversal (headkidney and subpharyngeal region) or sagittal (pituitary gland) were prepared for microscopical examination. The following staining procedures were used: Masson's trichrome method, including Ponceau de xylidine (Culling 1957); periodic acid–Schiff (PAS)–Orange G (Culling 1957); Bargmann's chrome–alum hematoxylin–phloxin (Pearse 1961); lead–hematoxylin (MacConaill 1947).

Results

During the experiment the control fish did not undergo visible changes. Their colour remained sea-green, as that of sexually immature fish. Androgen treatment induced the red skin colour typical of sexually mature fish. Six of the nine males killed after 4 weeks of either 11-ketotestosterone or 17α-methyltestosterone injections displayed this colour. Those killed after 7 weeks of androgen treatment all showed spawning coloration as well as hooked snouts and premaxillary teeth (Fig. 1). Estrogen treatment in the females also led to the appearance of an external red colour, and after 8 weeks the skin in these females was indistinguishable from that of normal sexually mature females. At the beginning of the study all fish fed actively but during the last few weeks a number of the androgen- and estrogen-injected fish showed irregularities in their food consumption.

Interrenal tissue

In the gonadectomized control fish, the interrenal tissue resembled that of sexually immature sockeye salmon. It consisted mostly of groups of densely packed small cells with slightly basophil cytoplasm and spherical chromatin-rich nuclei with inconspicuous nucleoli (Fig. 4). Few small blood sinusoids were present. Only in six of 28 controls, however, was this tissue homogeneously inactive. In others, some cell groups were found that showed signs of cellular hypertrophy: enlarged cells lacking cytoplasmic basophilia and often containing numerous small vacuoles, large nuclei, and conspicuous nucleoli. In two terminal control fish, both females, the hypertrophy was strong and extensive, involving more than half of the tissue examined.

Because of the heterogeneous appearance of the interrenal tissue in controls as well as in hormone-injected salmon, it was impossible to calculate a representative average nuclear diameter. Instead, a semiquantitative method was developed to estimate the histological activity of this tissue. Each fish was given a degree of hypertrophy according to the following scale: (1) tissue uniformly consisting of groups of densely packed small cells with basophil cytoplasm; (2) in general, same as (1), but small groups of hypertrophied cells present; (3) considerable cellular hypertrophy, but involving less than half of the tissue; (4) hypertrophy extensive, involving more than half of the tissue; (5) hypertrophy general, but small groups of normal quiescent cells still present; (6) all tissue uniformly hypertrophied. Table 1 shows the results of this analysis and gives an indication of the range of activity for each group of fish.

Effect of androgens

Injection of 11-ketotestosterone induced interrenal hypertrophy. The effect was weak in fish treated for 4 weeks but pronounced after 7 weeks. In all fish of group 2, the interrenal tissue was well vascularized. Five out of eight fish in the group had small hemorrhages. 17α-Methyltestosterone exerted a similar, but stronger effect. In this instance too, the induced hypertrophy of the interrenal cells was most pronounced in the fish treated for 7 weeks. Occasional hemorrhages were observed after 4 weeks of hormone injection, but after 7 weeks these were abundant (Fig. 5). In the last group, areas of degenerating cells with pyknotic nuclei were found.

Effect of estrogens

In the estradiol-injected fish interrenal hypertrophy was considerable, but it overlapped with that in the controls (Fig. 6, Table 1). Numerous blood sinusoids were present and the connective tissue had proliferated. Only one fish exhibited hemorrhages. A characteristic of this group was the presence of many mitotic figures. Indeed, this was the only group where mitotic activity was observed. Cell division in hypertrophied interrenal tissue of normal maturing sockeye salmon is rarely seen. In fish treated with estradiol cypionate the interrenal hypertrophy was more pronounced than in the previous group but there was less proliferation of connective tissue. In four out of the seven fish hemorrhages were present.

Effect of cortisol

The effect of cortisol, in general, was opposite to that of the gonadal hormones. In both males and females the interrenal tissue was slightly atrophic (Fig. 7, Table 1). In this respect, no differences were found between the sexes or between fish treated for 4, 7, or 8 weeks.

THYROID GLAND

In all fish, the thyroid gland follicles, extending along the ventral aorta, varied widely in size. An area of aggregated small follicles was present immediately adjacent to the large vessels (aorta and branchial arteries), whereas the larger ones were found scattered periferally throughout the loose connective tissue of the subpharyngeal area. In the control fish the histological features were those of an inactive gland (Fig. 8). The follicle epithelium was mostly squamous. The cytoplasm was basophil and in most cells contained one large rod-shaped PAS-positive inclusion and occasionally a few coarse granules. As a rule, the nuclei were flat, oriented parallel to the basement membrane, often indented or kidney shaped or sometimes lobated (Fig. 10). Their chromatin was evenly dispersed and there were no conspicuous nucleoli. Mitotic figures were not observed. The colloid in the follicle lumina was uniformly stained, acidophil, and strongly PAS-positive. Vacuoles were few in number and confined to the periphery of the colloid. Inactivity was most pronounced in the large follicles, which often showed an epithelium resembling a connective tissue capsule.

A distinct effect was obtained from treatment with androgens. In males injected with both 11-ketotestosterone and 17α-methyltestosterone, the larger follicles were not different from those in control fish. The aggregations of small follicles, however, showed marked signs of histological activation (Fig. 9). The cells of these last follicles were columnar and often protruded into the lumen. Their cytoplasm stained only weakly and contained a number of granular, weakly PAS-positive inclusions of variable size, mostly near the apex. The nuclei were large, invariably constricted into several lobules, and their orientation was usually perpendicular to the basement membrane (Fig. 11). The follicle contents were highly vacuolated and this reduced the PAS-positive colloid to small amounts in the center. In many small follicles it was even absent. In several instances the follicles were compressed and their epithelium folded.

The above features were more pronounced after 7 weeks of treatment with either androgen than

TABLE 1. Effects of androgens, estrogens, and cortisol on the interrenal tissue of gonadectomized sockeye salmon (*O. nerka*).

Group	Sex	Treatment	Duration (*weeks*)	Frequency distribution regarding interrenal hypertrophy in individual fish[a]						Occurrence of hemorrhages[b]
				(1)	(2)	(3)	(4)	(5)	(6)	
1	M	Controls	0	3	1	2	–	–	–	–
1	M	Controls	4	–	2	2	–	–	–	–
2	M	11-Ketotestosterone	4	–	1	1	2	–	–	+
3	M	17α-Methyltestosterone	4	–	–	2	2	1	–	+
4	M	Cortisol	4	4	2	–	–	–	–	–
1	M	Controls	7	1	2	3	–	–	–	–
2	M	11-Ketotestosterone	7	–	–	–	2	2	–	+
3	M	17α-Methyltestosterone	7	–	–	–	–	2	3	++
4	M	Cortisol	7	1	1	–	–	–	–	–
5	F	Controls	0	2	3	–	–	–	–	–
5	F	Controls	8	–	3	2	2	–	–	–
6	F	Estradiol	8	–	–	2	6	–	–	±
7	F	Estradiol cypionate	8	–	1	–	2	2	2	+
8	F	Cortisol	8	5	1	–	–	–	–	–

[a] For description of degrees 1–6, see text.

[b] – absent; ± occasionally observed; + common; ++ abundant.

after 4 weeks. No difference in effect between the two hormones could be found.

Estradiol or estradiol cypionate treatment had no apparent effect on the structure of the thyroid follicles; the follicles in fish injected with cortisol, males as well as females, were slightly more atrophic than those of the controls.

PITUITARY GLAND

Control fish

The pituitary glands of gonadectomized male controls exhibited the characteristics of fish castrated in an early stage of sexual maturation (McBride and van Overbeeke 1969a). Typical was the absence of granulated gonadotrophs (Fig. 2). Indeed, globular basophils (cells that contain PAS-positive inclusions with a strong affinity for light green), the predominant cell type in the pars distalis of mature sockeye salmon (van Overbeeke and McBride 1967), were noted in only five out of 16 control fish. They were found in small numbers, from 2 to 15 cells per midsagittal section, located in the ventrorostral part of the proximal pars distalis and occasionally among the prolactin cells of the rostral pars distalis. Dominating the proximal pars distalis were the acidophil cells, generally present in large numbers and stained a deep red with Masson's trichrome technique. With this last method a number of cells could be distinguished whose cytoplasm remained unstained. First, chromophobe cells (celltype 7, van Overbeeke and McBride 1967) located in the periphery of the dorsal cell columns, were noted in six fish. They were large and had a large spherical nucleus that was poor in chromatin but commonly contained a conspicuous nucleolus. Their cytoplasm, usually weakly PAS-positive and sometimes containing a fine, dustlike, PAS-positive granulation, frequently showed signs of vacuolar disintegration.

Common in all controls and more abundant than these last chromophobes was a cell type that, on the basis of its reaction to gonadectomy, has been considered a gonadotroph (McBride and van Overbeeke 1969a). The cells of this type were invariably small and present throughout the proximal pars distalis although they were most abundant in an area adjacent to the rostral pars distalis. Their nuclei were ellipsoid or elongate, sometimes angular, usually indented, rich in chromatin, but lacking nucleoli (Fig. 12). Similar cells, singly or in small groups, were found among the follicles in the rostral pars distalis. Although the cytoplasm of these cells was refractory to most dyes, the PAS-technique revealed a fine granulation in some of them.

In female controls the structure of the pituitary gland did not show any appreciable difference

Explanation of Figures 1–15

FIG. 1. Androgen-induced nuptial coloration in male sockeye salmon. Both fish were gonadectomized in July and killed in November. The upper one is an untreated control fish, and the lower fish was injected with 11-ketotestosterone for a period of 7 weeks.

FIG. 2. Mid-sagittal section of pituitary gland of gonadectomized male sockeye salmon (control). Photograph shows ventro-rostral part of the proximal pars distalis (to the right) and adjacent part of the rostral pars distalis (follicles to the left). Groups of cells with unstained cytoplasm and elongated nuclei (in center) represent gonadotrophs. Note absence of basophilia (light green) in these cells. Fuchsinophil cells (to the right) are growth hormone cells. Masson trichrome. ×400.

FIG. 3. Pituitary gland of gonadectomized male sockeye salmon treated with 17α-methyltestosterone for 7 weeks. Area shown is comparable with that of Fig. 2. Note the presence of well-granulated gonadotrophs in the proximal pars distalis as well as among the follicular cells of the rostral pars distalis. Compare with Fig. 2. Masson trichrome. ×400.

FIG. 4. Interrenal cells of gonadectomized female sockeye salmon (control). Cells are small and closely packed in strands. Masson trichrome. ×250.

FIG. 5. Hypertrophied interrenal tissue in gonadectomized male sockeye salmon, treated with 17α-methyltestosterone for 7 weeks. Note hemorrhages (arrows) and histolysis in the cores of the cellstrands. Masson trichrome. ×160.

FIG. 6. Interrenal cells of gonadectomized female sockeye salmon, injected with estradiol for 8 weeks. Areas of greatly enlarged cells contrast with cell groups that are showing only moderate hypertrophy. Note large nucleoli and mitosis (arrow). Compare with Fig. 4. Masson trichrome. ×250.

FIG. 7. Interrenal cells of gonadectomized female sockeye salmon, injected with cortisol for 8 weeks. The condition of these cells is similar to that in the controls. Compare with Fig. 4. Masson trichrome. ×250.

FIG. 8. Aggregation of small thyroid follicles in gonadectomized male sockeye salmon (control). The histological features suggest low activity of the gland. PAS-hematoxylin. ×160.

FIG. 9. Thyroid follicles of gonadectomized male sockeye salmon treated with 17α-methyltestosterone for 7 weeks. Area shown is comparable to that in Fig. 8. Note signs of heightened activity. Compare with Fig. 8. PAS-hematoxylin. ×160.

FIG. 10. Higher magnification of area shown in Fig. 8. Note squamous thyroid epithelium cells and deeply stained colloid. PAS-hematoxylin. ×1000.

FIG. 11. Higher magnification of area shown in Fig. 9. Thyroid epithelium cells are columnar and their nuclei are greatly enlarged, and the colloid is reduced. Compare with Fig. 10. PAS-hematoxylin. ×1000.

FIG. 12. Gonadotrophic cells in the pituitary gland of gonadectomized male sockeye salmon (control). Cells are small and contain chromatin-rich, often indented nuclei. Note absence of granulation. Masson trichrome. ×1000.

FIG. 13. Gonadotrophs in the pituitary gland of gonadectomized male sockeye salmon, treated with 11-ketotestosterone for a period of 7 weeks. Area shown is comparable with that in Fig. 12. Cells are enlarged and their cytoplasm is well granulated. Some cells contain coarse globules as well (arrow). Compare with Fig. 12. Masson trichrome. ×1000.

FIG. 14. Corticotrophic cells in the pituitary gland of gonadectomized male sockeye salmon (control). The layer of corticotrophs is seen between follicles of the rostral pars distalis (top of photo) and unstained projection of the neurohypophysis. Their cytoplasm contain fine, deeply stained granules. Lead–hematoxylin. ×400.

FIG. 15. Corticotrophs in the pituitary gland of male sockeye salmon treated with cortisol for 7 weeks. Area shown is comparable with that of Fig. 14. Note shrinkage of cells and loss of granulation. Compare with Fig. 14. Lead–hematoxylin. ×400.

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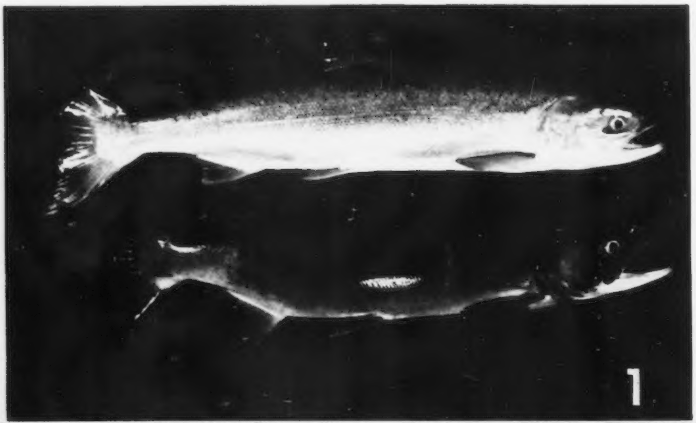
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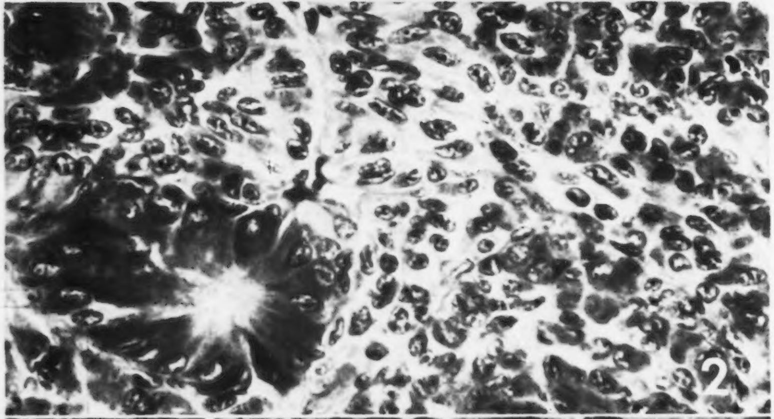
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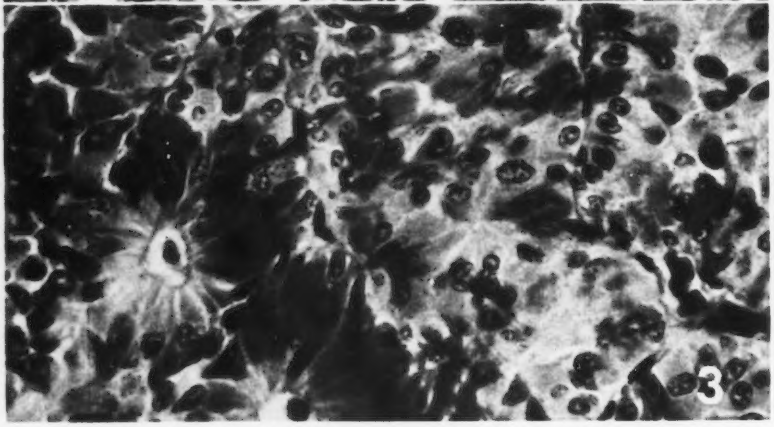
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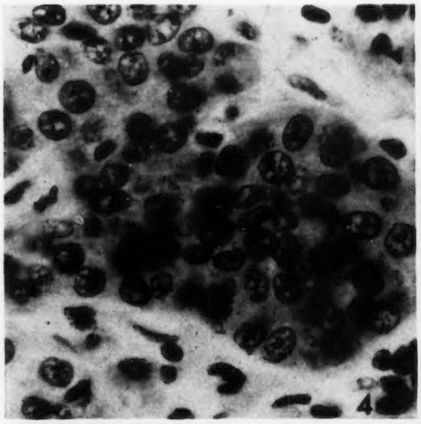

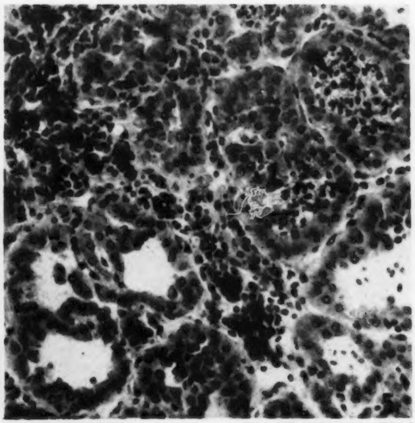

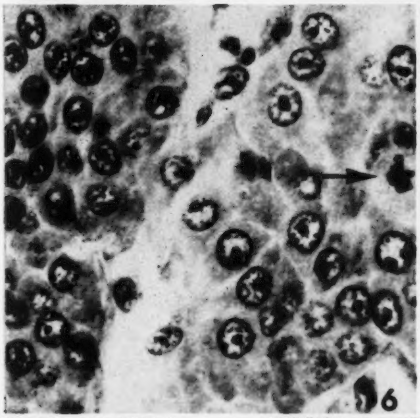

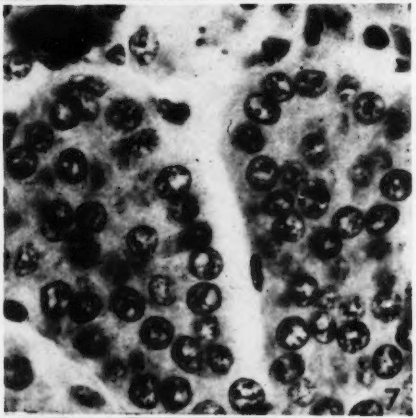

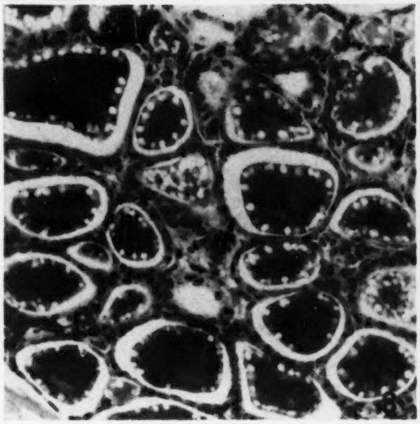

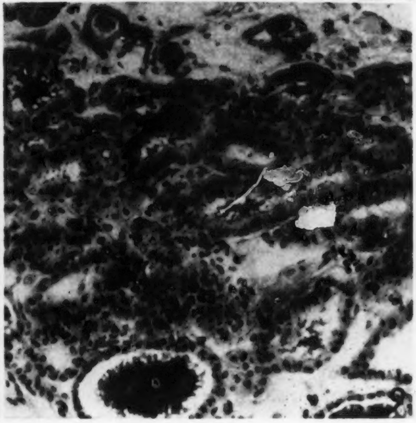

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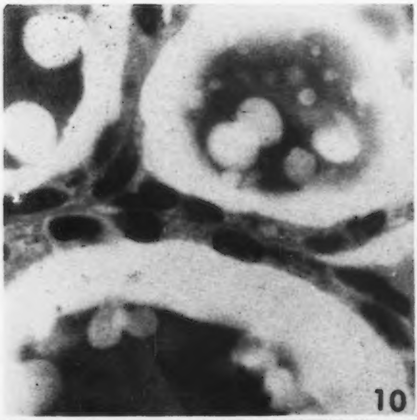

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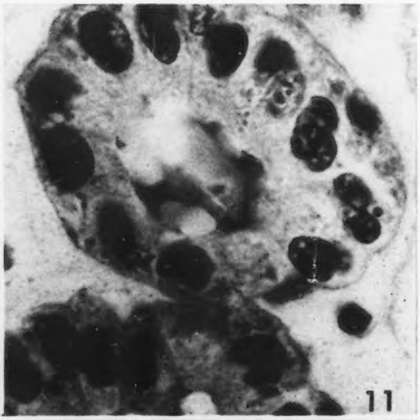

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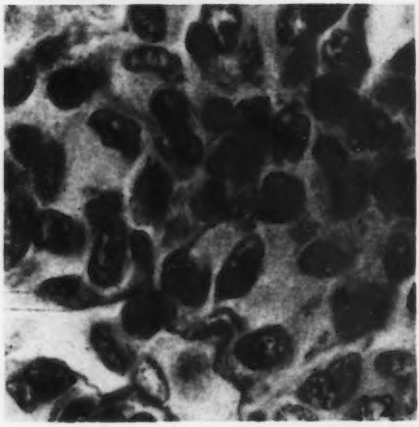

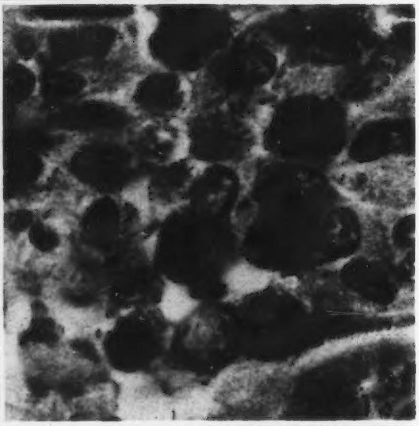

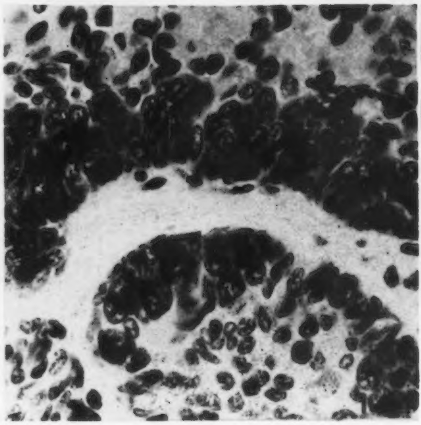

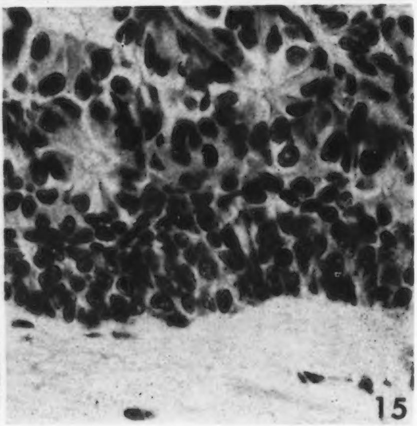

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from that in the control males. They too clearly showed the effect of gonadectomy but the reduction in basophilia (as compared with normal mature sockeye salmon) was somewhat less complete. In five out of 12 controls globular basophils were completely absent. In the remainder, basophils were found but their number did not exceed 20–25 per midsagittal section. In these females too, gonadotrophs were agranular and appeared to be abundant in a zone adjacent to the rostral pars distalis. During the period of hormone treatment the histological structure of the pituitary gland in the controls did not undergo any visible change.

Effect of androgens

A striking effect of administration of androgens was the appearance of granulated basophil cells. Four weeks after the commencement of 11-ketotestosterone injections, part of the gonadotrophs contained numerous small, light green-stained, PAS-positive granules. Such cells were present in the rostral pars distalis and in the adjoining area of the proximal pars distalis (Fig. 3). No basophils were noted in the four control fish sampled at this time. Continuation of the treatment for an additional 3 weeks led to a slight increase in basophilia. Although no visible increase in the number of basophils was evident, the cells did exhibit a heavier granulation and some of them, notably those located among the prolactin cells of the rostral pars distalis, contained coarse globules as well. In two of the four fish treated for a period of 7 weeks, granulated basophils were also observed in the deeper part of the proximal pars distalis, along the border with the pars intermedia. Accumulation of granules in the gonadotrophs was accompanied by an increase in cell size (Fig. 13). Furthermore, the nuclei were slightly larger in the granulated cells and their nucleoli were more conspicuous. The unstained gonadotrophs did not differ visibly from those in the control fish. The effect of 17α-methyltestosterone was stronger than that of 11-ketotestosterone. Four weeks of 17α-methyltestosterone injections evoked a basophilia that was indistinguishable from that produced by 7 weeks, treatment with 11-ketotestosterone. Continued administration of 17α-methyltestosterone for another 3 weeks resulted in a further increase in abundance of basophil cells. In each of the five fish in this last group, basophils were present throughout both parts of the pars distalis although their largest concentrations were still found near the rostral pars distalis. Moreover, many cells contained some large globules in addition to small granules and frequently the cytoplasm itself was PAS-positive and deeply stained with light green. The nuclei were idented and occasionally cup shaped. Unstained agranular gonadotrophs, although reduced in abundance, were still present in all fish.

In most androgen-injected fish, large chromophobe cells were observed but the proportion of these cells fluctuated widely. Vacuolar degeneration of the cytoplasm in these cells was commonly noted. Neither the abundance nor the structure of the chromophobes appeared to be affected by androgen treatment. Furthermore, no apparent effect could be detected on the corticotrophs in any of the treated fish.

Effect of estrogens

Estradiol administration to females during 8 weeks produced an effect that was similar to that induced by 17α-methyltestosterone in males within 7 weeks. Basophil cells were, however, slightly more abundant and their inclusions were generally somewhat coarser. Estradiol cypionate treatment induced changes in the basophils of the pars distalis that, although being of the same kind, were much weaker and did not exceed those brought about in males by 11-ketotestosterone injected for a period of 4 weeks. The corticotrophs in these females were not visibly affected.

Effect of cortisol

Corticotrophic cells in the control fish could be readily recognized after staining with lead hematoxylin. They were mostly columnar and the cytoplasm contained a fine dustlike black granulation (Fig. 14). Their nuclei were from 7.5 to 9.5 μ long and ranged in form from ellipsoid and slightly idented to kidney shaped.

Although the intensity of the granulation varied somewhat between individual fish, no differences were noted between the various groups of control fish or between males and females.

Cortisol treatment resulted in pronounced changes in these cells. Particularly evident was the loss of granulation (Fig. 15). Also, the corticotrophs in the cortisol-injected fish were much smaller than those in the controls as indicated by the more densely aggregated nuclei. The nuclei had undergone a reduction in size and were from 5.0 to 8.0 μ and their nucleoli were usually inconspicuous. The above effect of cortisol injection appeared to be more pronounced in males than in females.

Discussion

The absence of clear hypertrophy of the interrenal tissue in gonadectomized control fish at the beginning of the experiments, confirms our earlier observation

(McBride and van Overbeeke 1969b) that gonadectomy of sockeye salmon in an early stage of sexual maturation prevents the development of hyperadrenocorticism. Comparison of the condition of this tissue in the various control groups, both males and females, indicates that during the period that peanut oil was administered a slight activation occurred. This is probably the result of stress involved in the handling of the fish during injections.

The result of our investigations, that androgen- and estrogen-treated fish developed morphological characteristics of interrenal hyperfunction, is consistent with the fact that in these same fish the adrenocortical homologue showed increased physiological activity, as reported by Fagerlund and Donaldson (1969) and Donaldson and Fagerlund (1969). The results, furthermore, support the idea, first proposed by Hane et al. (1966), that hyperadrenocorticism accompanying sexual maturation in Pacific salmon is caused by gonadal hormones rather than by metabolic demands related to the buildup of gonad tissue. The strongest effect was produced by 17α-methyltestosterone in males. Fagerlund and Donaldson (1969) also found that 17α-methyltestosterone was more effective than the salmon's natural androgenic hormone 11-ketotestosterone. In the medaka (*Oryzias latipes*), 17α-methyltestosterone was found to be 100 times more androgenic than 11-ketotestosterone (Arai 1967; Egami and Arai 1965). Whether the effects of gonadal hormones and their derivatives observed in the present study are mediated by changes in ACTH production remains to be investigated. In an earlier report we tentatively suggested the possibility of a direct effect (McBride and van Overbeeke 1969b). This suggestion was based on the absence of a direct response of the pituitary corticotrophic cells to gonadectomy in mature salmon. The fact that the corticotrophs were not visibly affected by injection of gonadal steroids either, as noted in the present investigation, lends further support to this suggestion.

Several investigations indicate that Pacific salmon during the later part of sexual maturation exhibit a marked reduction in thyroid gland function (Robertson and Wexler 1960; Robertson et al. 1961a; McBride 1967; Chestnut MS 1970). In sockeye salmon that had been induced to resume feeding, this reduction appeared to be partly prevented (McBride 1967). This observation on the effect of feeding was confirmed in the present study. The structure of the thyroid follicles in the control fish indicated inactive glands but the involution of the epithelium was less pronounced than in normal sexually mature and spawning sockeye salmon and the epithelial thickness showed a greater variation. It is surprising that the restorative histological effect of the two androgens tested was limited to the small follicles. The question arises whether the observed hypertrophy included cell-proliferation or even formation of new follicles. The absence of any mitotic figures would suggest that this was not the case. However, amitotic cell divisions could have occurred, but since the nuclei were highly lobated, the possible occurrence of amitosis could not be ascertained.

Effects of androgenic steroids on the thyroid gland in fish have been reported by several workers. Matty (1960) noted thyroid hypertrophy in *Sparisoma squalidum* in response to testosterone phenylacetate and methyltestosterone. Singh (1968) found that testosterone caused an increase in iodine uptake in *Mystus vittatus*. He subsequently showed that this effect was not mediated by the pituitary gland (Singh 1969). It should be added that this last investigator noticed that other steroids, viz. estrogen, progesterone, and cortisone, exerted a similar influence (Singh 1969). In the salmon, we did not find an effect of estrogen and cortisol on the thyroid gland. Recently, Sage and Bromage (1970a) reported that 17α-methyltestosterone stimulated the thyroid in *Poecilia reticulata*, and their work also suggests that this effect is probably a direct one, not involving the pituitary gland. The physiological significance of the effect of androgens on the thyroid in sockeye salmon remains to be clarified. There are no indications that any such effect takes place during sexual maturation, at which time the thyroid normally undergoes progressive atrophy. Therefore, the observed experimental effects may be pharmacological rather than physiological.

It has been shown that the pituitary–interrenal relation in sockeye salmon involves a negative feedback mechanism (Fagerlund et al. 1968; Fagerlund and McBride 1969). The pronounced reaction of the corticotrophs to cortisol injections observed in this study constitutes further support for such a relation. That the corticotrophs responded with degranulation and atrophy, however, may be surprising inasmuch as one would expect cortisol to suppress the release of ACTH and, consequently, to increase the granulation. Initially, such a reaction may well have taken place, but our analysis is essentially that of a long-term effect and the over-all involution of the tissue would seem to be in accordance with a reduced ACTH-producing function of the pituitary gland. The slight atrophy of the interrenal tissue in the cortisol-treated fish also indicated a reduced activity of the pituitary–interrenal axis.

The marked differentiation of granulated basophils, following androgen and estrogen administration, was unexpected, since there are now sufficient indications for the existence in teleosts of a negative feed-back control of gonadotrophic activity by gonadal steroids, similar to that in higher vertebrates (Egami and Ishii 1962; Ball and Baker 1969; Sage and Bromage 1970b). That the increased basophilia reflected activation of the cells seems unquestionable: although the accumulation of granules in itself might be considered a consequence of inhibited release, it is difficult to see how such an inhibitory action could lead to an increase in cell size and, in particular, to enlargement of the nuclei and activation of nucleoli. These results, therefore, are in contrast with those of Sage and Bromage (1970b) who noted that androgen treatment of *Poecilia reticulata* resulted in progressive atrophy and general loss of stainability of the gonadotrophs. This last investigation, however, differed from our study in at least two important respects. First, Sage and Bromage investigated effects of androgens on normal gonadotrophs in intact mature fish, whereas the sockeye salmon we used were gonadectomized and their gonadotrophs were undifferentiated. Furthermore, Sage and Bromage made their analysis after 3–24 days, whereas in our study, samples were examined after 4 and 7 weeks.

The question arises whether among the newly differentiated basophils in the hormone-treated sockeye salmon there were any thyrotrophs. That gonadal hormones may have an effect on thyrotrophs in fish was recently shown by Sage and Bromage (1970a). However, they found the effect of several androgens, including methyltestosterone, to be inhibitory. As we reported earlier (McBride and van Overbeeke 1969a), in adult salmon thyrotrophs are probably scarce, if present at all, but the possibility cannot be excluded that some were present and that their response to steroid treatment was similar to that of the gonadotrophs.

Acknowledgments

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Effects of Androgens, Estrogens, and Cortisol on the Skin, Stomach, Liver, Pancreas, and Kidney in Gonadectomized Adult Sockeye Salmon (*Oncorhynchus nerka*)

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McBride, J. R., and A. P. van Overbeeke. 1971. Effects of androgens, estrogens, and cortisol on the skin, stomach, liver, pancreas, and kidney in gonadectomized adult sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 28: 485–490.

A study was made of the histological changes occurring in the skin, stomach, liver, pancreas, and kidney of adult gonadectomized male and female sockeye salmon in response to hormone treatments. The males received 11-ketotestosterone, 17α-methyltestosterone or cortisol for 4 or 7 weeks, and the females received estradiol, estradiol cypionate, or cortisol for 8 weeks.

In the males, androgen injection evoked a highly significant increase in the thickness of the epidermis, a marked atrophy of the stomach, and a degeneration in the liver and kidney. In the pancreas, the exocrine portion showed characteristics of cytolysis in the acini, whereas the islets of Langerhans were hypertrophied. These changes were generally more pronounced after 7 weeks than after 4 but, in the skin, no further increase in the thickening of the epidermis was noted after the 4th week of treatment. No differences between responses of these tissues to 11-ketotestosterone and 17α-methyltestosterone were detected.

Estrogen administration in the females evoked similar, albeit weaker, responses in the skin, stomach, pancreas, and kidney than those recorded in the androgen-treated males. The liver of the females exhibited the characteristics of a hyperactive organ, which probably reflects estrogen-induced vitellogenesis.

The effects of cortisol were similar in both sexes: little, if any, change was noted in the skin; atrophy or degeneration was observed in the stomach, liver, kidney, and in the exocrine portion of the pancreas, and the islets of Langerhans were hypertrophied. The cortisol-induced alterations were not, however, as pronounced as those noted in either the androgen- or estrogen-treated fish.

These changes are discussed in relation to the changes observed in normal sexually maturing and reproducing Pacific salmon.

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The changes that occur in the Pacific salmon during sexual maturation and reproduction can be divided into two categories. First, there are those directly associated with the reproductive function: gonad maturation, development of secondary sexual characteristics, and changes in behavior. The second category concerns a complex of alterations, partly of a degenerative nature, occurring in a variety of organs and tissues, notably in some of the endocrine glands, the gastrointestinal tract, and the cardiovascular system. The exact relation between these two categories is unknown. On the one hand, the influence of the gonads may well extend beyond the reproductive activity. It was shown by Idler et al. (1961) that 11-ketotestosterone, identified as an androgen in sockeye salmon, not only brings about the nuptial coloration in this species and the typical elongation of the snout in males but, in addition, induced an increase in thickness of the skin and a loss of red muscle pigmentation. Also, McBride et al. (1963), using the same species, found that gonadectomy of mature fish led to a reversal of the external secondary sexual characteristics, as well as a prolongation of the life span well beyond the time when these fish would normally have spawned and died. Confirmation of these last results was recently obtained by McBride and van Overbeeke (1969a, b) and van Overbeeke and McBride (1971). Moreover, it was observed that gonadectomy of adult sockeye salmon in an early stage of sexual maturation prevents the development of deteriorative alterations in the skin, alimentary tract, pancreas, and kidneys (McBride and van Overbeeke unpublished data). Investigations by Robertson and his co-workers,

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however, indicate that many of the changes of the second category are to be associated with the hyperadrenocorticism that develops at this stage of the life cycle of the genus *Oncorhynchus* (Robertson and Wexler 1960; Robertson et al. 1961a, b, 1963).

The present communication is part of an investigation concerning the role of the gonad-interrenal axis in the development of the complex of changes mentioned above. It describes the histophysiological changes that occur in the skin, liver, stomach, kidney, and pancreas after the administration of androgens, estrogens, and cortisol to gonadectomized sockeye salmon. Other results of this investigation, namely the effect of these hormones on the distribution and secretion of cortisol, as well as on the pituitary gland, interrenal tissue, and thyroid gland, were reported earlier (Fagerlund and Donaldson 1969; Donaldson and Fagerlund 1969; van Overbeeke and McBride 1971).

Materials and Methods

As the conditions under which these fish were captured and held in captivity, as well as the experimental procedures followed, have been described (van Overbeeke and McBride 1971), only a brief outline is given.

The fish used in this study were adult sockeye salmon, gonadectomized in June 1967, while still sexually immature. For several months after the operation, the fish were held under a natural photoperiod and were fed. Fish showing a complete absence of secondary sexual characteristics were then selected for this study. Due to limited aquarium facilities, the effects of hormone injections in males and females were studied in separate experiments with a time interval of approximately 5 weeks.

Males — In October 1967, the gonadectomized males were divided into four groups: group 1 (10 fish) served as controls; group 2 (8 fish) received 11-ketotestosterone; group 3 (10 fish) was injected with 17α-methyltestosterone; and group 4 (8 fish) received cortisol. All injections were intramuscular and given twice weekly. Per injection, the fish received 2.50 mg hormone dissolved in 0.2 ml of peanut oil. Controls received 0.2 ml of peanut oil. Six controls were killed at the beginning of the study. At the end of the 4th week, samples of fish were killed as follows: group 1, four fish; group 2, four fish; group 3, five fish; and group 4, six fish. The remainder were killed at the end of the 7th week.

Females — In January 1968, the gonadectomized females were divided into four groups for treatment as follows: group 5 (12 fish) served as controls; group 6 (eight fish) received estradiol; group 7 (eight fish) was given estradiol cypionate; and group 8 (six fish) was injected with cortisol. Estradiol and cortisol were administered twice weekly in amounts of 2.50 mg dissolved in 0.2 ml of peanut oil. Estradiol cypionate was injected once a week in a dosage of 1.0 mg dissolved in cotton seed oil. Five control fish were killed at the beginning of the experiment. The other seven controls received 0.2 ml peanut oil twice weekly. All injections were intramuscular and were continued for a period of 8 weeks.

Histological techniques — Fish were immobilized by severing the spinal cord just caudal to the head, and samples of liver, kidney, stomach, and pancreas were immediately excised and placed in Bouin-Hollande-Sublimate fixative. Samples of skin were taken from a point midway between the dorsal fin and the lateral line and fixed together with the other tissues in the same fixative. After 24 hr of fixation, the skin samples were decalcified in 5% HNO_3 in 70% ethanol for 4 hr. All tissues were dehydrated, cleared, and embedded in Paraplast (mp 56 C) and sectioned at a thickness of 5 μ. Sections were stained with Mayer's hematoxylin and eosin or with Masson's trichrome method (Culling 1957). To differentiate between the α and β cells in the islets of Langerhans, sections of the pancreas were stained with Gomori's aldehyde fuchsin (Pearse 1961).

Results

When the injections were begun, the external features of the fish were indistinguishable from those of sexually immature sockeye prior to gonadectomy. In the control fish, the external appearance did not show any visible change during the period of injections. Similarly, the histological structure of their skin, stomach, liver, pancreas, and kidney did not exhibit any alterations. These results confirm our earlier observation that the external sea-green coloration of the adult sockeye is maintained if the fish are gonadectomized while sexually immature (McBride and van Overbeeke unpublished data). In contrast, the fish that received steroid hormones exhibited profound changes, not only in their external features, but also in the various organs listed above.

Skin — The integument of sockeye salmon consists of two parts: the epidermis, an outer stratified squamous epithelium, and the dermis, an inner layer of connective tissue. The dermis can be divided into a relatively thick superficial layer of loose tissue ("reticular zone") that also contains the scales, and a deeper layer of densely packed fibres, the stratum compactum.

In the control fish, at the beginning of the experiment, the epidermis was a layer of three to six cells, and the germinal cells were cuboidal to low columnar (Fig. 3). During the experiment, a slight thickening of the epidermis occurred in both sexes. Injection of the two androgenic compounds induced a marked increase in thickness of the skin. The epidermis, as well as both layers of the dermis, was involved in the response, but it was most conspicuous in the former. In the androgen-injected fish, the germinal layer consisted of tall columnar cells, whereas the epithelium itself was from 12 to 18 cells thick. Mucous cells were present throughout the epidermis and no longer confined to the surface layer of cells (Fig. 4). The superficial dermal connective tissue was more diffuse than that in the controls. No differences were noted between the effect of 11-ketotestosterone and that of 17α-methyltestosterone. In both instances, the effect of 7 weeks' treatment was not notably stronger than that of 4 weeks.

In the hormone-injected fish, the boundary between the stratum compactum and the underlying muscle and fat was often not distinct. For this reason, and also because the thickness of the epidermal epithelium appeared to be more constant, measurements were made of the latter. The mean thickness of the epidermis was determined from four measurements, taken at different sites of the skin sample of each individual fish. The results concerning the males are shown in Fig. 1. Statistically significant differences were found between the group injected with 11-ketotestosterone and the comparable controls after 4 weeks ($P < 0.001$) and after 7 weeks ($P < 0.001$), as well as between the 17α-methyltestosterone-injected group and the controls after 4 weeks ($P < 0.001$) and after 7 weeks ($P < 0.001$). No differences were found between the two experimental groups.

Estrogen injection in the females brought about changes in the skin that were of the same quality as those evoked in the males by androgens, but they were much less pronounced (Fig. 2). Both estradiol and estradiol cypionate evoked a statistically significant increase in thickness of the epidermis ($P < 0.001$). However, the response in the estradiol-injected fish was stronger than that in the estradiol cypionate-treated salmon ($P < 0.001$).

Administration of cortisol in the males led to a small but statistically significant increase in the thickness of the epidermis that was noticeable after 4 weeks of treatment ($P < 0.01$), but no longer after 7 weeks. In the females, the skin of the cortisol-injected fish was indistinguishable from that in the controls.

Stomach — In the control fish, the stomach exhibited the characteristics of actively feeding salmon. The mucosal layer had numerous villi. Its epithelium consisted of columnar cells with large elongated nuclei, containing conspicuous nucleoli. Gastric glands were abundant and active (Fig. 5). Cells of the stratum granulosum, an irregular zone of acidophil granular cells found in the deeper part of the mucosa, were small and sparse and they were only moderately granulated. Both the circular and the longitudinal part of the zona muscularis were well developed.

In males treated with 11-ketotestosterone or 17α-methyltestosterone for 4 weeks, slight changes were noted. All fish showed a reduction in size of the gastric villi; some exhibited a more general but moderate atrophy. The effect of 7 weeks of androgen administration was much stronger. Atrophy of the villi was pronounced and general, and in a few fish, villi were virtually absent (Fig. 6). The lining epithelium was much lower than that in the controls, although the individual cells had maintained their columnar shape (Fig. 7). The gastric glands were visibly shrunken and showed signs of degeneration. The thickness of the zona muscularis was reduced to less than half of that in the controls. A striking effect was noticed in the stratum granulosum. The cells of this zone had undergone a marked proliferation and were large and heavily granulated. No difference could be detected between the effect of 11-ketotestosterone and that of 17α-methyltestosterone. Estrogen administration evoked the same response. Although no differences in the effect were observed between the two estrogens used, their effects seemed to be somewhat weaker than that of the androgens.

A still less-marked effect was observed in the fish treated with cortisol. In males injected for a period of 4 weeks, the only clear response was a reduction of the epithelium cell height. Atrophy of the mucosa, as well as of the stomach musculature, was evident, however, in the terminal samples of both sexes. The stratum granulosum in these fish exhibited a mixed response. In a few fish, the cells were large and well granulated; in others, they were small and contained few granules. Although these cells were more abundant in the cortisol-treated fish than in the controls, their numbers were definitely smaller than those in either the androgen- or the estrogen-injected fish.

Liver — The livers of the uninjected control fish were not different from those in normal, actively feeding, sexually immature sockeye salmon (Fig. 8). The most striking result of the hormone administration was the difference between the structure of this organ in the males treated with androgens, and that in the females injected with estrogens. Androgen administration induced vacuolization of the cytoplasm, which was general after 4 weeks. After 7 weeks, there was a loss of cytoplasm and disintegration of nuclei, as well as general disorganization of the tissue (Fig. 9). These degenerative features are the same as those observed in spawning salmon. No differences in effect between 11-ketotestosterone and 17α-methyltestosterone could be detected. On the other hand, the livers of the estrogen-injected females did not show any signs of deterioration. On the contrary, they exhibited hypertrophied cells containing finely granulated cytoplasm and clearly enlarged nuclei with conspicuous central nucleoli (Fig. 10). Mitotic figures were seen in the tissue of the estradiol-treated females, but not in that of the estradiol cypionate-injected fish. In the livers of the cortisol-treated fish, both males and females, some cellular degeneration, mostly a loss of cytoplasm and occasional pyknosis of the nuclei, had occurred, but the deterioration was restricted to small areas. In this respect, no differences were found between treatment of 4 weeks and 7 weeks in the males.

Pancreas — In adult sockeye salmon, the pancreas is found in intimate association with the pyloric caeca and the adipose omentum. The endocrine part, the islets of Langerhans, is embedded in the exocrine pancreas. In the controls, the islets were generally spherical, from 75 to 100 μ in diameter (Fig. 11). Both the α and β cells were well granulated. Also, the acinar cells of the exocrine pancreas showed histological signs of activity. Scattered throughout the tissue complex were groups of large fat cells, belonging to the omentum. Androgen treatment resulted in hypertrophy and hyperplasia of the islets of Langerhans, many of which attained a diameter in excess of 400 μ (Fig. 12). This process involved both the α and β cells, but the granulation of these cells did not visibly differ from that in the controls. The exocrine pancreas exhibited widespread deterioration. Most cells were devoid of granules, and nuclear as well as cytoplasmic disintegration was general. Furthermore, typical fat cells were virtually absent from this tissue Although these features could be recognized in all fish after 4 weeks, whether injected with 11-ketotestosterone or 17α-methyltestosterone, the extent of the effect at this time was not without considerable variation. After 7 weeks, the effects were stronger, in particular the tissue degeneration. The response in the females to administration of estradiol and estradiol cypionate was essentially the same as that observed in the males treated with androgens for 7 weeks. The effect of cortisol was, in general, not unlike that of the other hormones, but it was less drastic and evident only in the terminal samples. In these fish, islet hypertrophy was obvious, but the cells of the exocrine pancreas showed only a moderate reduction in granulation, and degeneration of the acini was found in one fish only. Also, depletion of fat cells was incomplete.

Kidney — The most conspicuous effect of both androgens and estrogens on the kidney was glomerular dilation, accompanied by sclerosis of glomerular capillaries and a thickening of Bowman's capsules (Fig. 13 and 14). Whereas edematous glomeruli were only occasionally noted in a few of the control fish, they were common in all fish treated with these hormones for 7 or 8 weeks. These fish also exhibited a loss of cytoplasm from the tubular cells. Only a slight effect was noted in males treated with androgens for 4 weeks. Four weeks of cortisol administration had no visible effect on the kidney structure but, after 7 weeks (males) or 8 weeks (females), the effect of this hormone was comparable to that of 4 weeks of androgen injection.

Discussion

The results of this investigation, that androgens and estrogens when administered to gonadectomized sockeye salmon bring about changes in various tissues similar to those normally taking place in sexually maturing salmon, indicates that these last changes are a response to gonadal hormones rather than to general stress associated with gonadal growth and starvation. The question arises whether these alterations are mediated by the enhanced interrenal function. Robertson et al. (1963), on the basis of their study on the effect of hydrocortisone in immature rainbow trout, concluded that "the principal histological changes characteristic of the spawning salmon are caused by the abnormally-high concentration of hydrocortisone and cortisol in the blood." An exception was the skin, which did not respond to hydrocortisone by hyperplasia but, reportedly, underwent a slight atrophy. In our study, cortisol caused a slight thickening of the skin in males, but this was significant only in the fish injected during a period of 4 weeks. The absence of an effect of cortisol after 7 weeks and 8 weeks in males and females, respectively, together with the fact that a slight increase in skin thickness occurred in the controls of both sexes, suggests that the effect observed in the males after 4 weeks of cortisol administration may be attributed to stress, probably due to handling of the fish during injections. On the other hand, the pronounced response of the skin to androgens and estrogens clearly indicates that, in normal maturing salmon, the thickening of the skin is caused directly by gonadal hormones. It is interesting that the observed effect was much stronger in the males than in the females. Robertson and Wexler (1960) reported that in spawning salmon, the skin of the males is 25% thicker than that of the females. Idler et al. (1961) found that in maturing sockeye salmon, the effect of 11-ketotestosterone on the skin is less pronounced in females than in males. Whether the difference that we observed between males and

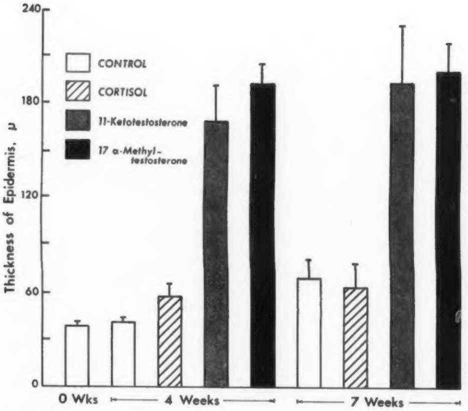


FIG. 1. Effects of 4 and 7 weeks' administration of cortisol, 11-ketotestosterone, and 17α-methyltestosterone on the thickness of the epidermis in adult gonadectomized male sockeye salmon.

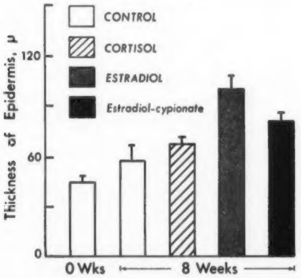


FIG. 2. Effects of 8 weeks' administration of cortisol, estradiol, and estradiol cypionate on the thickness of the epidermis in adult gonadectomized female sockeye salmon.

Explanation of Figures 3–14

FIG. 3. Skin of a gonadectomized control male sockeye. The epidermis is a layer of 3–5 cells thick. The cells of the germinal layer are cuboidal. Masson trichrome. ×80.

FIG. 4. Epidermis of a gonadectomized male sockeye administered 11-ketotestosterone for 7 weeks. Note the marked increase in the thickness of the epidermis and the columnar cells of the germinal layer. Compare with Fig. 1. Masson trichrome. ×80.

FIG. 5. Portion of the stomach of a gonadectomized control male sockeye. Note well-developed villi and prominence of gastric glands (arrows). Masson trichrome. ×40.

FIG. 6. Area of stomach comparable with that shown in Fig. 5 of a gonadectomized male sockeye injected with 17α-methyltestosterone for 7 weeks. Villi are visibly reduced and gastric glands are virtually absent. Masson trichrome. ×40.

FIG. 7. A, Higher magnification of the stomach epithelium shown in Fig. 5. Cells are tall and nuclei exhibit conspicuous nucleoli. Masson trichrome. ×250. B, Higher magnification of the stomach epithelium shown in Fig. 6. Cells and their nuclei are much smaller than those shown in Fig. 7A. Masson trichrome. ×250.

FIG. 8. Strands of liver cells of a gonadectomized control female sockeye. Hematoxylin and eosin. ×250.

FIG. 9. Liver cells of a gonadectomized male sockeye administered 17α-methyltestosterone for 7 weeks. Note disintegration of cytoplasm and nuclei. Compare with Fig. 8 and 10. Hematoxylin and eosin. ×250.

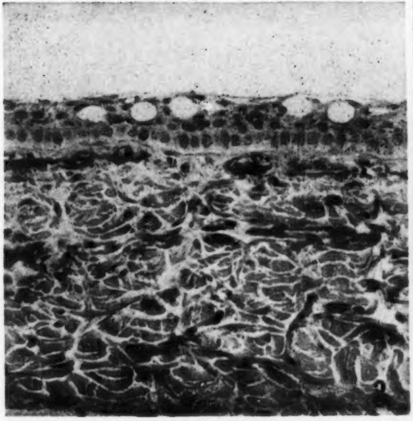
FIG. 10. Liver cells of a gonadectomized female sockeye injected with estradiol for 8 weeks. Cells visibly hypertrophied with nuclei showing conspicuous nucleoli. Compare with Fig. 8 and 9. Hematoxylin and eosin. ×250.

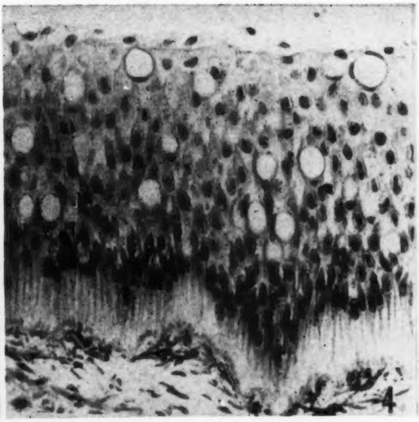
FIG. 11. Pancreas of a gonadectomized control male sockeye. Two small islets of Langerhans (arrows) are embedded in the dark-coloured exocrine tissue. Lower half of the photograph shows numerous fat cells of the omentum. Masson trichrome. ×65.

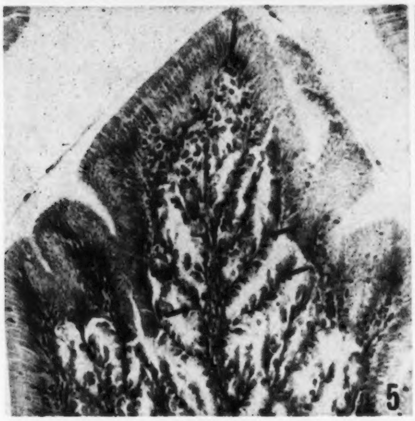
FIG. 12. Pancreas of a gonadectomized male sockeye administered 11-ketotestosterone for 7 weeks. Note the two hypertrophied islets of Langerhans (arrows) and the absence of fat cells. The exocrine tissue is in a state of degeneration. Compare with Fig. 11. Masson trichrome. ×65.

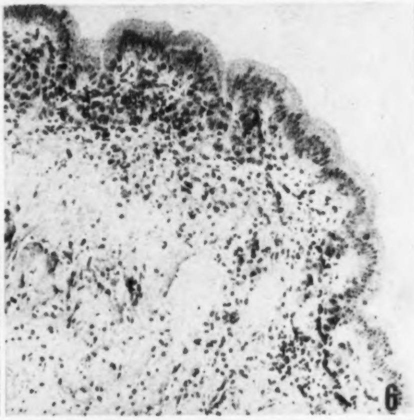
FIG. 13. Kidney of a gonadectomized control female sockeye showing a normal glomerulus. Masson trichrome. ×200.

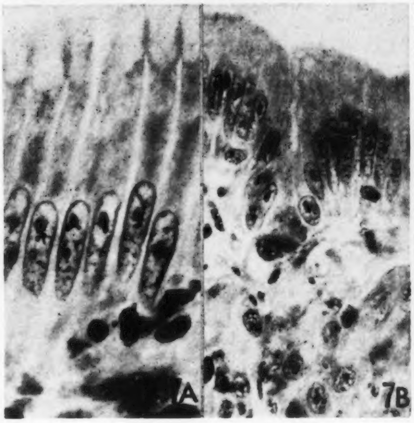
FIG. 14. Kidney of a gonadectomized female sockeye administered estradiol cypionate for 8 weeks showing an edematous glomerulus. Note compression of the glomerular tuft and capillary sclerosis. Masson trichrome. ×200.

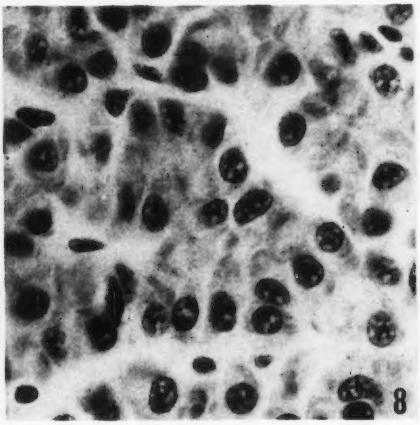












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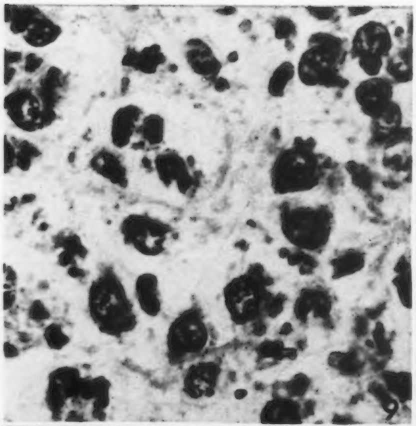

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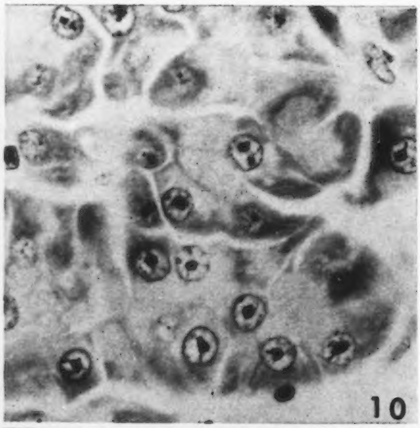

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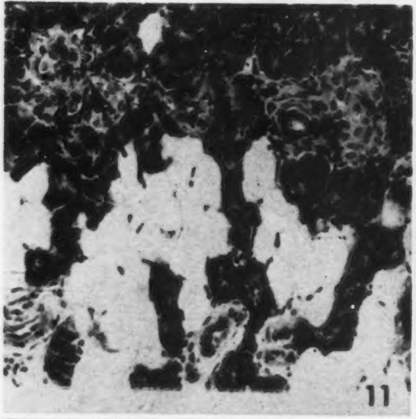

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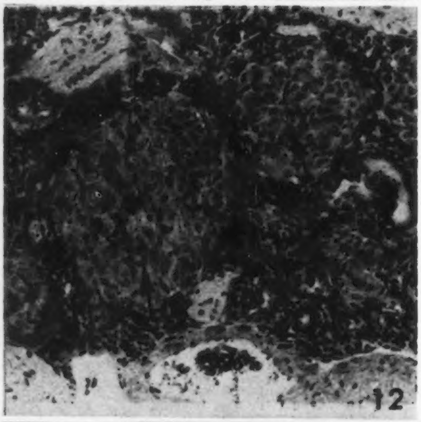

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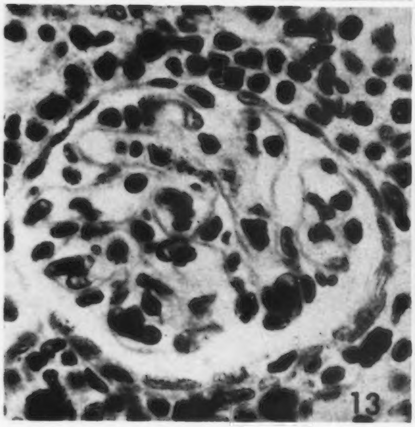

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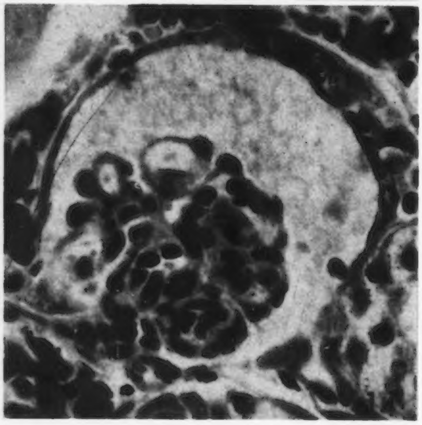

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females is to be ascribed to a sex difference in responsiveness of the integument, or reflects differences between the hormones used, remains to be investigated.

The effect on the liver seems to be a complex one. The two androgens and, to a lesser extent cortisol, produced the same degenerative changes as those noted in sexually maturing Pacific salmon of both sexes (Robertson and Wexler 1960; McBride et al. 1965) as well as those evoked in rainbow trout by hydrocortisone treatment (Robertson et al. 1963). But the liver structure in the estrogen-treated females clearly indicates a hyperfunction of this organ. Not only is there a marked difference in condition of the liver between the 2 sexes, but in this respect, the estrogen-injected females also differ sharply from normal maturing females. The observed effect of estrogens on the liver may be explained as follows. Several investigations have shown that in teleosts, as in other vertebrates, the liver is involved in vitellogenesis (Seal and Doe 1969). That this process is controlled by estrogenic hormones is supported by observations in coho salmon, showing that a serum lipoprotein fraction, presumably serum vitellin, associated with egg formation, was found only in maturing females. In sockeye salmon, this same compound could be induced by administration of estradiol monobenzoate in both males and females (Ho and Vanstone 1961; Vanstone and Ho 1961). Similarly, Hodgins and Utter (1967) showed that in sockeye salmon, the presence of a lipoprotein antigen (SM factor), apparently produced by the liver, was closely related to sexual maturation. Indirect evidence for an estrogen-controlled vitellogenic role of the liver in oviparous teleosts stems from investigations by Kobayashi (1953), using *Misgurnus anguillicaudatus*, and by Egami (1955), studying *Oryzias latipes*. In these two species, a sex difference exists during the breeding season, the liver in females being more active than that in males. Idler and Tsuyuki (1958) reported that in sockeye salmon during maturation, females have significantly larger livers than males, but, to our knowledge, no sex-linked difference in histological structure of the liver has been recorded. In the fish used in this study, yolk deposition in the eggs was impossible and, consequently, an accumulation of vitellin probably occurred. The estrogen-induced hypertrophy of the liver cells, therefore, may reflect an enhanced yolk production together with an inhibited release of this product.

The hormone-induced degenerative alterations in the stomach, the exocrine pancreas, and the kidney are the same as those observed in sexually maturing salmon. They were generally most pronounced in the androgen-injected fish but, otherwise, did not differ from the changes brought about by estrogens and cortisol. Therefore, no conclusion can be drawn as to whether or not these changes were mediated by the interrenal tissue in the fish treated with sex hormones. Also, the observation that gonadal hormones as well as cortisol-induced marked hypertrophy of the islets of Langerhans, does not permit identification of the direct cause of the islet hyperplasia in normal maturing salmon. Robertson and his co-workers related islet hyperplasia to the elevated corticosteroid production (Robertson et al. 1961a, 1963).

Acknowledgments

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Cardiac and Respiratory Responses to Hypoxia in the Sea Raven, *Hemitripterus americanus*, and an Investigation of Possible Control Mechanisms

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Responses of the sea raven (*Hemitripterus americanus*) to hypoxia consisted of an increase in rate and amplitude of breathing and an increase in dorsal and ventral aortic pressure. Changes in heart rate seldom occurred. Bilateral transection of cranial nerves IX and X resulted in a fall in dorsal aortic PO_2 to a sustained low level but did not alter the dorsal and ventral aortic blood pressure or breathing responses induced by hypoxia.

Pressure changes in the opercular cavity during breathing seldom exceeded .5 cm H_2O and rarely exceeded 1 cm H_2O under extreme hypoxia. There were no obvious differences in opercular pressure wave forms before and after bilateral section of nerves IX and X.

An artificial "heart–gill" machine was devised with which blood PO_2 could be altered outside the fish and the blood perfused into either the dorsal or ventral aorta. Breathing amplitude was increased by perfusing blood of low PO_2 into the dorsal aorta. While maintaining dorsal aortic PO_2 high and constant, increases in breathing amplitude were induced by low ambient PO_2 levels.

Vasomotor changes in blood pathways other than the gills probably contribute to the changes in blood pressure observed during hypoxia. Preliminary evidence for both central and peripheral sites of oxygen receptor activity is provided.

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CARDIAC and respiratory reflexes induced by hypoxia are well documented in teleosts (Hughes and Shelton 1962; Shelton and Randall 1962; Randall and Shelton 1963; Holeton and Randall 1967a, b; Randall and Smith 1967; and Marvin and Heath 1968) and elasmobranchs (Satchell 1959, 1960, 1962; Baumgarten-Schumann and Piiper 1968; and Piiper et al. 1970). These studies have given indirect evidence that certain cardiac and respiratory responses involve chemoreceptors on or near the gills where environmental oxygen levels could be monitored. Afferent fibers from the chemo-sensitive carotid and aortic glomi of mammals reach the respiratory center via cranial nerves IX and X. In fish these nerves innervate the gills, pseudobranch, and palatine organ of the roof of the mouth. However, there is little information about such chemoreceptor activity in fishes. Although Laurent (1967) recorded oxygen chemoreceptor activity in branches of cranial nerve IX from the pseudobranch of the tench, the physiological role of this structure in cardiac and respiratory reflexes is unknown. DeKock (1963) found large numbers of "end-buds" well exposed to the respiratory flow in the pharyngeal cavity of brown trout and salmon. These potential chemoreceptors do not resemble typical chemoreceptive tissue as found in the aortic bodies of higher vertebrates, and although Konishi et al. (1969) observed high CO_2 sensitivity in the palatine, facial, and branchial nerves that probably innervate taste buds in the carp, these receptors were insensitive to changes in PO_2. Similarly, altering PO_2 did not influence the level of spontaneity in branchial branches of cranial nerves IX and X in the sea raven (Sutterlin and Saunders 1969).

The gills and branchial pump musculature of fishes are innervated by branches of cranial nerves V, VII, IX, and X. The bilateral section of nerves V and VII stops regular breathing movements through destruction of the motor supply to the branchial pump musculature (Hughes and Shelton 1962). Several studies have been made of the effects of bilateral section of nerves IX and X in teleosts (Powers and Clark 1942; Shelton 1959; and Hughes and Shelton 1962) and in elasmobranchs (Springer 1928; Satchell 1959, 1961). Hughes and Shelton (1962) report that the tench shows more or less normal breathing responses

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(increased amplitude of mouth and opercular movements) to low PO_2 and high PCO_2 levels after bilateral section of the IXth and Xth nerves close to the brain. They state that nerves IX and X have no obvious function in supplying any part of the pumping apparatus since in most instances their bilateral section or branchial portions thereof usually altered the breathing rhythm but did not abolish breathing.

The present study was undertaken to make extensive observations of cardiac and respiratory responses to hypoxia in unanesthetized fish before and after nerve transection and thereby to elucidate any chemoreceptor reflexes that could regulate and integrate cardiac and respiratory activity.

Materials and Methods

The Atlantic sea raven (*Hemitripterus americanus*) was used because of its large size, its large ventral aorta, the relative ease of cannulating the ventral and dorsal aortae, and the ability of the fish to withstand extensive surgery. Fish were trawl-captured in Passamaquoddy Bay, N.B., and held in a 6-ft diam fiberglass tank of running sea water. During experiments, December to April, water temperature in the holding tank was from 2 to 4 C. All experiments were done within this range. Fresh herring was offered weekly, but few fish fed at the low temperatures. Sea ravens used for experiments were 45–55 cm long and weighed from 1 to 3 kg. Other specimens were used as a source of whole blood for transfusion and perfusion. There were no obvious immune reactions to the introduction of "foreign" blood.

The experimental plan required that blood pressures and PO_2's be measured in the dorsal and ventral aortae and that these measurements sometimes be made in conjunction with artificial perfusion of these blood vessels with either oxygenated or deoxygenated blood before and after sectioning cranial nerves IX and X.

OPERATIVE PROCEDURES

Cannulation of ventral aorta — For experiments requiring measurement of ventral aortic pressure and PO_2 but no artificial perfusion, a T-shaped cannula (16 cm of 1.5 mm I.D. polyethylene tubing joined to 1 cm of 3.7 mm I.D. tubing) was used. The fish was anesthetized in M.S. 222 and placed ventral side up on an operating table. The skin and musculature of the isthmus connecting the opercula ventrally were cut forward along the midline for about 6 cm. The ventral aorta was exposed by cutting the skin to one side of the midline. Connective tissue was cleared by blunt dissection from about 4 cm of the aorta forward of the bulbus. An incision was made in the ventral aorta and the T-cannula filled with heparinized Ringer solution (Hudson 1968) was inserted into the aorta and tied in place. One or two ml of heparinized Ringer were injected into the fish through the cannula. The skin was sewn tightly around the cannula to prevent entry of sea water. Gill perfusion was not performed during the operation because of the danger of flooding exposed tissues with sea water but was started as soon as the incision was closed. In most instances the fish began breathing within a few minutes after being put into a recovery tank.

During experiments in which the fish was perfused with blood of different O_2 content, a loop cannula was substituted for the T-cannula (Fig. 1). The cannula (40 cm of 3.7 mm I.D. polyethylene tubing heat bent in U-shape) was secured to the cut ends of the ventral aorta. Thus, blood pumped by the heart was diverted outside the fish through this cannula and then back into the ventral aorta and into the normal circulatory path. After observations of hypoxic responses with the loop intact, the loop was cut to make connections for the aortic perfusion. The loop circuit could be reestablished after experiments and the fish left in an aquarium to recover. Fish with U-cannulae survived several days and appeared to breathe normally.

Cannulation of dorsal aorta — Cannulation of the dorsal aorta was done by sliding 1.5 mm I.D. polyethylene tubing (about 6 cm) into a gastric artery to a point previously shown by dissection to be at the junction of that vessel and the dorsal aorta (Fig. 1). The artery was exposed by making a 6-cm incision in the body wall to the right of the pelvic fin. Heparinized Ringer was injected as soon as the cannula was tied in place, after which the incision in the body wall was sewn up.

Cannulation of opercular cavity — A cannula of 1.5 mm I.D. polyethylene tubing was inserted in the opercular cavity (Saunders 1961). This was used in conjunction with a pressure transducer and pen recorder for measuring rate and amplitude of breathing.

Sectioning cranial nerves — Anesthetized fish that had been cannulated previously were held upright on the operating table and the gills were irrigated with sea water. The skin and muscle over the region of the medulla were folded back and a section of cranium about 1 cm square was removed from the soft, semi-transparent brain case. Some cerebro-spinal fluid was aspirated to improve visibility in the brain case. Cautery usually stopped bleeding from small dermal blood vessels. The IXth or Xth nerves, or both, were bilaterally sectioned about 3 mm from the brain. The skin and muscle were replaced over the opening and secured with Braun Histoacryl tissue adhesive and the fish was held overnight in an aquarium to recover.

EXPERIMENTAL PROCEDURE

The fish was held in a darkened plexiglass container supplied with water in which the rate of flow and PO_2 were regulated (Fig. 1). Saline-filled cannulae from the ventral and dorsal aortae and opercular cavity were connected to differential pressure transducers (Sanborn, Model 268B). The PO_2 of blood and water was measured using a Beckman macro O_2 electrode in a glass cuvette maintained at the same temperature as the fish bath. Hydrostatic pressures in the aortae and in the opercular cavity and PO_2 were recorded on a Beckman Type R Dynograph recorder that was some-

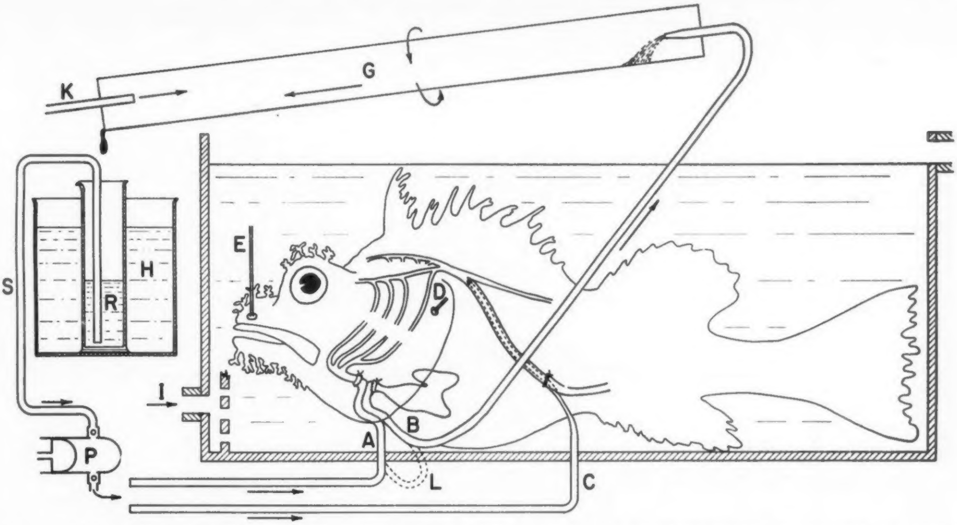

FIG. 1. Diagram of blood perfusion apparatus. During ventral aortic perfusion, loop cannula L connecting cannula A to B is removed, blood is pumped by fish through cannula B directly into reservoir R bypassing artificial gill G. Blood is perfused by pulsatile pump P through cannula A into fish. Pressure in opercular cavity is measured in cannula D. Blood pressure is measured in cannula B giving heart rate, in cannula C giving dorsal aortic pressure, and in cannula A giving ventral aortic or perfusion pressure. Blood PO_2 in reservoir R is measured in tubing S and dorsal aortic PO_2 is measured in cannula C. Inspired PO_2 of water is measured in cannula E. H is ice bath and K is tube for delivery of air or N_2 gas into artificial gill. I is inlet for oxygenated or deoxygenated water. During dorsal aortic perfusion, cannula A is clamped. Blood is pumped by fish through cannula B into the rotating artificial gill and then pumped through cannula C into dorsal aorta. Blood pressure is measured in cannula B giving heart rate and in cannula C giving perfusion pressure. Blood PO_2 in reservoir R is measured in tubing S and inspired PO_2 of water is measured in cannula E. Opercular pressure is measured in cannula D.

times coupled to a Grass Model 7 Polygraph recorder run at a slower speed to compress records of experiments lasting up to 8 hr.

Blood perfusion — Experiments were conducted to study the effects of hypoxia on the above mentioned parameters while regulating blood perfusion rate and PO_2 levels. During ventral aortic perfusion, the loop cannula was interrupted and the blood pumped by the heart of the fish was led through a tube terminating in a small orifice (Fig. 1). The height of the tube and the diameter of the orifice were adjusted so that the heart was pumping against a constant hydrostatic head and ventral aortic blood and pulse pressures were maintained at normal levels. Blood was collected in a graduated cylinder and then pumped without changing its PO_2 into the ventral aorta by a pulsatile blood pump, incorporating variable stroke and speed (Model 1405, Harvard Apparatus Company).

An artificial "heart–gill" apparatus was used to alter the PO_2 of the blood and to pump it back into the dorsal aorta of the fish (Fig. 1). This apparatus consisted of a 2.6-cm I.D. glass tube 50 cm long, mounted at an angle and rotated by a variable speed motor, together with the blood pump described previously. Blood pumped by the heart of the fish, as described previously, was led into the "gill." A thin film of blood coated the sides of the tube, and a counter-current of air or N_2 was passed through the tube to effect blood gas exchange. Blood was collected in a chilled graduated cylinder from which it was pumped into the dorsal aorta of the fish in the same manner as for ventral aortic perfusion.

About 50–100 ml of heparinized blood from other sea ravens were used to fill the perfusion pump and associated tubing and to keep the system operating when the fish's cardiac output was insufficient to keep some blood in the reservoir. The various parameters were measured during experimentally induced hypoxia before the perfusion apparatus was connected, and then with the "heart–gill" apparatus connected.

Results

HYPOXIC RESPONSES — CRANIAL NERVES INTACT

Typical hypoxic responses of sea ravens with cranial nerves intact include an increase in rate and amplitude of breathing movements and an increase in ventral and dorsal aortic blood pressure (Table 1, Fig. 2). Data for all parameters were not always collected simultaneously as it was sometimes desirable to make some measurements with

TABLE 1. Comparison of the response to hypoxia before (no parentheses) and after (in parentheses) bilateral section of cranial nerves IX and X, +, an increase; –, a decrease; and 0, no change in the parameters indicated.

Response parameter	Fish												
	A	B	C	D	G	O	P	Q	R	S	T	U	V
Breathing rate	+ (+)	+ (+)	+ (0)	+ (0)	0	+	0 (0)	+	+	+ (+)			+ (+)
Breathing amplitude	+ (0)	+ (+)	+ (+)	+ (+)	+	+	+ (+)	+	+	+ (+)			+ (0)
Heart rate	0 (0)	– (0)	0 (–)	0 (0)		–	0 (0)	0			0	0	– (0)
Ventral aortic blood pressure (VA)	+ (+)	+ (+)	+ (+)	+ (+)		+	+ (+)	+			+	+	+ (+)
Dorsal aortic blood pressure (DA)			+ (+)	+				+				+	
Differential blood pressure (VA–DA)			+	0				0					
Dorsal aortic PO$_2$, *mm Hg* (normoxic conditions)			35–54 (4–23)	97–108 (14–23)	44–60	105–114	90–93 (6–9)	75–90	54–62				
Dorsal aortic PO$_2$, *mm Hg* (hypoxic conditions)			14–15 (3–4)	12–15 (0–3)	5–22	9–12	9–11 (3–6)	10–15	9–14				
Ventral aortic PO$_2$, *mm Hg* (normoxic conditions)			30–33 (4–9)	22–39 (5–10)				15–20					
Ventral aortic PO$_2$, *mm Hg* (hypoxic conditions)			15–17 (2–4)	12–14 (0–2)									

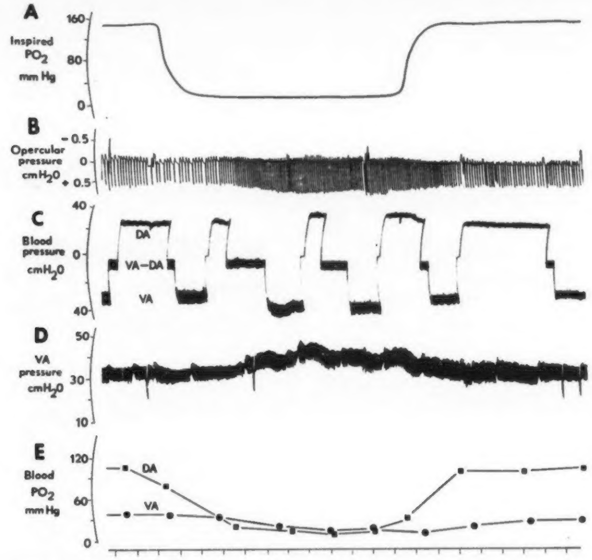

FIG. 2. Response to hypoxia of fish D with cranial nerves intact. Records A, B, and C taken simultaneously. Records D and E obtained during subsequent hypoxia treatments. DA and VA represent dorsal and ventral aorta respectively. VA–DA represents differential pressure. Time trace on abscissa indicates 1-min intervals.

limited surgical intervention; at other times measurements failed owing to technical difficulties.

Differential pressure between ventral and dorsal aortae often remained constant (Table 1). Heart rate usually remained constant, but occasionally a transitory bradycardia existed and is indicated as a decrease in heart rate (Table 1). Bradycardia usually did not persist throughout the period of hypoxia. The ambient PO_2 at which there were noticeable changes in the above parameters was about 30 mm Hg.

Dorsal and ventral aortic PO_2's from 50 to 100 and 15 to 40 mm Hg, respectively, were common in resting fish with cranial nerves intact. These values fell to the ambient level during extreme hypoxia. Dorsal aortic PO_2's were sometimes lower than those in the ventral aorta during hypoxia (Fig. 2, 4, and 5). Recovery of the above parameters to prehypoxic levels was usually complete within 3 min after restoration of normoxia.

HYPOXIC RESPONSES — CRANIAL NERVES IX AND X BILATERALLY SECTIONED

The most striking change after section of nerves IX and X was a marked drop in dorsal and ventral aortic PO_2 (Table 1). Figure 3 shows responses to hypoxia measured for the same fish the day after those in Fig. 2, and approximately 20 hr after bilateral section of cranial nerves IX and X. In all individuals so treated, dorsal and ventral aortic blood pressure and breathing responses to hypoxia were similar before and after nerve section.

Breathing responses to hypoxia were variable before and after nerve transection in that some individuals showed increased breathing rate and amplitude whereas others increased only one or the other of these parameters (Table 1). Bilateral section of nerves IX and X did not abolish hypoxic responses (increased rate or amplitude of breathing, or both). However, these fish seldom lived longer than 1 week and progressive deterioration was apparent from their behavior. Individuals recovering from nerve transection or resting between experiments breathed very irregularly and feebly, with periods of apnea often lasting as long as 1 min.

The amplitude of opercular pressures in resting unstressed sea ravens seldom exceeded 0.5 cm H_2O and rarely exceeded 1.0 cm under extreme hypoxia (Fig. 2–9). Sectioning cranial nerves IX or X, or both, did not alter these amplitudes. Moreover, there were no obvious differences in opercular pressure wave forms before and after nerve transection. The wave form consisted of a

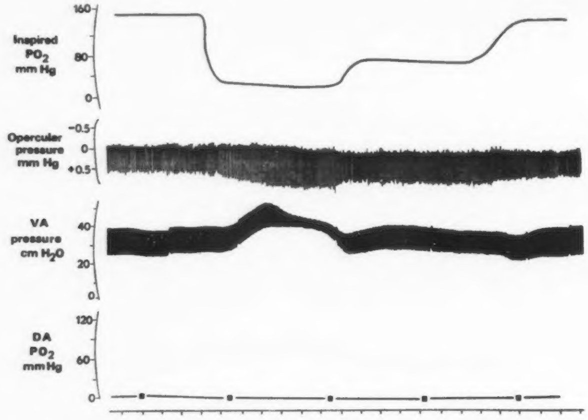

Fig. 3. Response of fish D to hypoxia 24 hr after section of cranial nerves IX and X. All records obtained simultaneously. Time: 1-min intervals.

prolonged suction phase produced by great expansion of the opercular chamber during opercular abduction, followed by a short positive pressure phase, resulting from opercular adduction. There was a marked increase in the magnitude of the suction phase during hypoxic breathing of both operated and unoperated fish. Although no measurements were made of respiratory volume, it was obvious from the turbulence near the opercular openings that this low pressure system moved a large volume of water.

HYPOXIC RESPONSES — CRANIAL NERVE IX BILATERALLY SECTIONED

The pronounced reduction in dorsal aortic PO_2 after section of nerves IX and X could have resulted from sectioning either nerves IX or X or both, or from the extensive operative procedures and removal of some cerebral spinal fluid from above the brain. Therefore, the above operative procedures were simulated on two fish on day 1. The skull was opened and closed but with no cranial nerves being cut. The fish was tested on day 2. Immediately after this, the skull was reopened and cranial nerve IX was bilaterally sectioned. The fish was retested on day 3.

Breathing and blood pressure responses to hypoxia of sham-operated fish (Table 2) were similar to those in unoperated fish (Table 1). Hypoxic responses of sea ravens with nerve IX bilaterally sectioned were similar to those for the same fish after the sham operation (Table 2). The dorsal aortic PO_2's of the two individuals, after both the sham operations and the bilateral section of nerve IX (Table 2), were within the range found for unoperated fish (Table 1). Recordings from one of the individuals (fish E) after the sham operation (Fig. 4) and after nerve IX was sectioned (Fig. 5) show similar hypoxic responses.

PERFUSION OF DORSAL AND VENTRAL AORTAE

To assess the effects of various ventral aortic perfusion rates on cardiac output, breathing, and dorsal aortic PO_2, two fish were subjected to a seven- to 10-fold increase in perfusion rate (Table

TABLE 2. Comparison of responses to hypoxia of two fish (E and F) after sham operation and again after bilateral section of cranial nerve IX. Symbols +, –, 0, as in Table 1.

	Sham-operated		Nerve IX sectioned	
Response parameter	E	F	E	F
Breathing rate	+	+	+	0
Breathing amplitude	+	+	+	+
Heart rate	0	0	0	0
Ventral aortic pressure	+	+	+	+
Dorsal aortic pressure	+	+	+	
Dorsal aortic PO_2 (normoxic), *mm Hg*	75–78	68–90	71–78	42–50
Dorsal aortic PO_2 (hypoxic), *mm Hg*	14–29	8–11	10–15	11–17

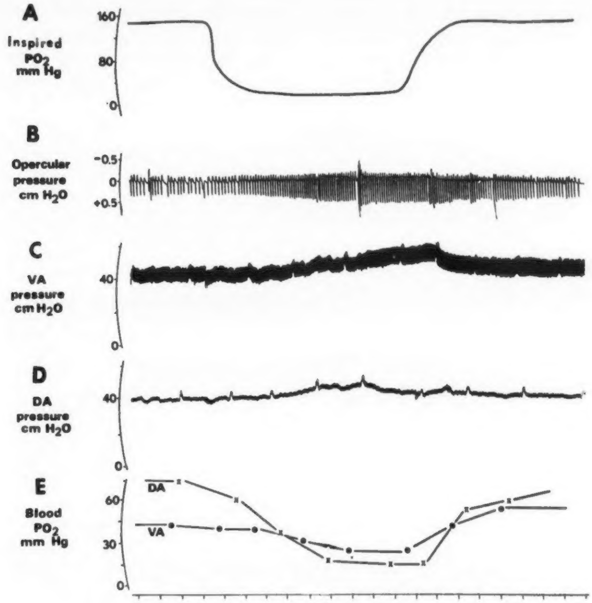

FIG. 4. Response of fish E to hypoxia 24 hr after sham operation. Records A, B, C, and dorsal aortic PO_2 in E obtained simultaneously. Record D and ventral aorta PO_2 in E obtained during subsequent hypoxic treatment. Periodic distortions in pressure record are associated with blood PO_2 sampling. Time: 1-min intervals.

3). At low perfusion rates, cardiac output of the fish was lowered until it equalled perfusion rate. As perfusion rate was increased stepwise, cardiac output similarly increased to the same values. At low perfusion rates the volume of blood in the fish decreased and at high perfusion rates the volume increased until perfusion rate and cardiac output became equal. In other words, within wide limits, the fish pumped blood at the same rate blood was pumped into it by simultaneously altering its blood volume and stroke volume.

Under these circumstances, changes in heart rate did not contribute to the increased cardiac output. Throughout an eightfold increase in perfusion rate, dorsal aortic PO_2 decreased only slightly. Breathing rate and amplitude were not affected appreciably. In the following experiments the dorsal and ventral aortic perfusion rates employed were those that did not result in any change in total blood volume in a fish resting in aerated water. For example, for fish M (Table 3), a perfusion rate between 4.5 and 7.3 ml/min would be chosen, and the stroke rate and volume of the perfusion pump would be adjusted accordingly to simulate the normal stroke volume and cardiac output of the fish.

Before blood perfusion was performed, the response of the fish to hypoxia was examined 24 hr after cannulation. The ventral aortic loop was left intact and the environmental PO_2 was lowered. The responses of three fish (O, P, R), tested in such fashion, are shown in Fig. 6. All fish showed normal dorsal aortic PO_2 values in aerated water and drastic reductions in PO_2 in hypoxic water. Breathing amplitude during hypoxia increased in all three fish; breathing rate increased in fish O and R. Ventral aortic blood pressure increased in fish P and R; it was not measured in fish O.

Ventral aortic perfusion with unaltered venous blood (technique shown in Fig. 1) was performed within 2 hr of procedures described above. Responses to environmental hypoxia similar to those in Fig. 6 were observed (Fig. 7). Although perfusion rate was kept constant during this experiment, blood pressure in the dorsal aorta and perfusion pressure increased in all three fish during hypoxia.

Following ventral aortic perfusion, the venous blood being pumped by the heart was directed into the aerating device (Fig. 1). The PO_2 of this blood was elevated to the desired level by regulating the rotation rate and air flow. Oxygenated blood was then perfused into the dorsal aorta at the same rate used previously during ventral aortic perfusion. Under these conditions, there could

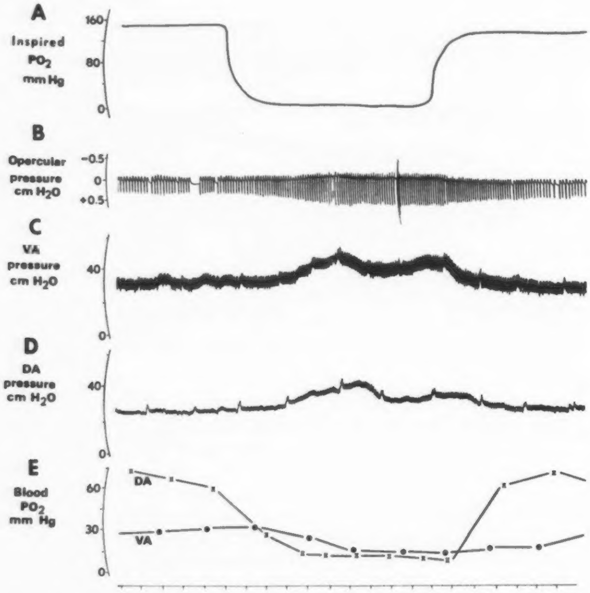

FIG. 5. Response of fish E to hypoxia 24 hr after bilateral section of cranial nerve IX. Records A, B, C, and DA PO_2 in E obtained simultaneously. Record D and VA PO_2 in E obtained during subsequent hypoxic treatment. Time: 1-min intervals.

TABLE 3. The effect of altering ventral aortic perfusion rates on various cardio-respiratory parameters in two fish, M and L. Venous blood was pumped by the heart to a chilled reservoir and pumped by a perfusion pump through a cannula to the ventral aorta. Cranial nerves were left intact. Stroke volume of the fish heart was obtained by dividing perfusion rate by heart rate during conditions when perfusion rate ≐ cardiac output.

Fish	Perfusion rate (*ml/min*)	Heart rate (*beats/min*)	Stroke volume (*ml*)	Δ blood volume (*ml*)	Breathing rate (*breathing movements/min*)	Breathing amplitude (*cm H_2O*)	Dorsal aorta PO_2 (*mm Hg*)	Ventral aorta PO_2 (*mm Hg*)
M	3.1	21	0.15	−3	8	0.5	125	9
	4.5	21	0.21	−1	9	0.5	135	9
	7.3	21	0.35	+5	8	0.5	135	10
	19	21	0.90	+11	8	0.6	120	10
	34	18	1.9	+27	8	0.6	84	13
L	4.2	22	0.20	−4	9	0.5	129	6
	8.4	22	0.38	−1	9	0.5	123	6
	14	22	0.64	+2	9	0.6	105	7
	18	22	0.82	+7	9	0.6	105	6
	24	22	1.1	+10	9	0.6	105	8
	30	21	1.4	+27	9	0.6	102	9

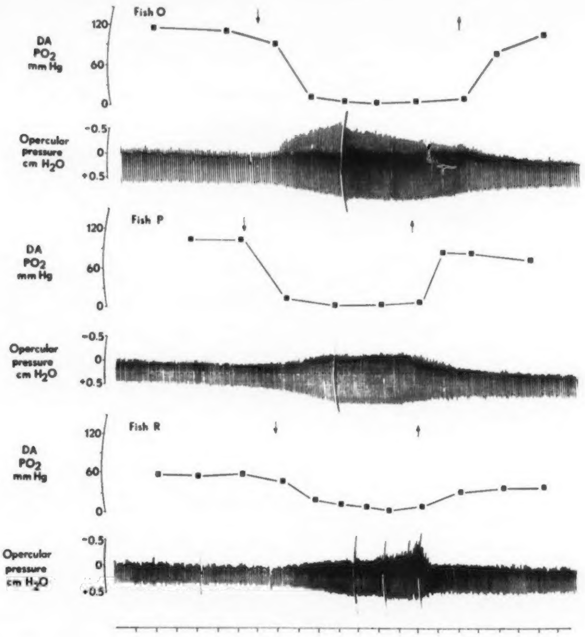

FIG. 6. Responses of three fish (O, P, R) to hypoxia 24 hr after loop cannula was inserted into ventral aorta. Arrows indicate beginning (↓) and end (↑) of deoxygenated water flow into fish chamber. Chamber PO_2 was approximately 150 mm Hg at the beginning and fell within 2 min to about 20 mm Hg. Within 2 min after second arrow, chamber PO_2 rose again to 150 mm Hg. Time: 1-min intervals.

be little or no blood flow through the gills. In three fish so treated the response to environmental hypoxia was an increase in breathing amplitude (Fig. 8), although the PO_2 of the perfusion blood, and hence of the dorsal aorta, did not vary appreciably. The responses of fish O and P, however, were of smaller magnitude and more delayed than those shown before (Fig. 6) and during (Fig. 7) ventral aortic perfusion.

In a variation of the above experiments N_2 was substituted for air in the "gill." The resulting deoxygenated blood was perfused directly into the dorsal aorta of several fish. The response was an increase in breathing amplitude (Fig. 9B). However, the magnitude of this response was never as great as that observed when the same fish was subjected to low environmental PO_2 during ventral aortic perfusion with unaltered venous blood (Fig. 9A). An increase in perfusion pressure during hypoxia was also observed during both ventral aortic and dorsal aortic perfusion (Fig. 9A and B).

Discussion

COMPARISON OF HYPOXIC RESPONSES OF SEA RAVEN AND OTHER TELEOSTS

Breathing rate and amplitude in sea ravens did not increase noticeably until ambient PO_2 fell below about 30 mm Hg, whereas in tench these hypoxic effects were evident at about PO_2 75–80 mm Hg (Randall and Shelton 1963) and in rainbow trout at about 140 mm Hg (Holeton and Randall 1967a). Although bradycardia is a normal hypoxic response in tench and rainbow trout, beginning at about 35–40 and 90 mm Hg respectively, it was not usually observed in sea ravens, even at PO_2's as low as 10 mm Hg. Blood pressures in both dorsal and ventral aortae of rainbow trout increased gradually as PO_2 fell below 155 mm Hg (Holeton and Randall 1967a). Such cardiovascular responses in the sea raven were not seen until PO_2 was reduced to 30 mm Hg or lower, levels that lead to respiratory and cardiac collapse in trout (Holeton and Randall 1967a).

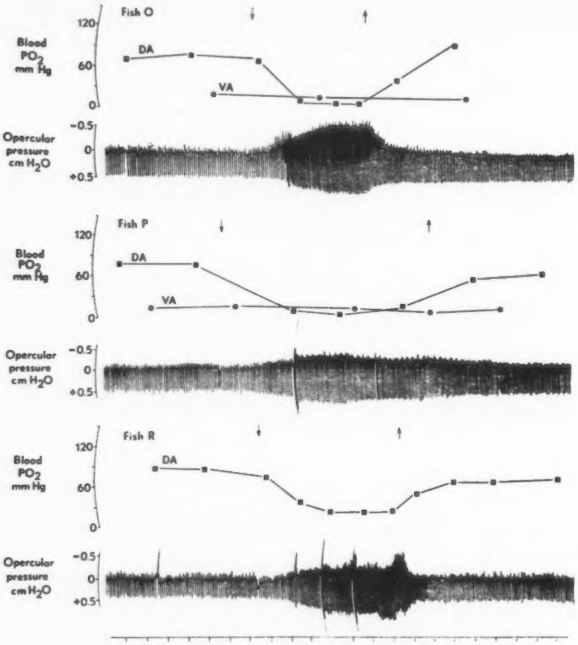

FIG. 7. Responses of three fish (O, P, R) to hypoxia during ventral aortic perfusion with unaltered venous blood. Arrows as in Fig. 6. Ventral aortic PO_2 was measured on blood before being pumped back into the ventral aorta. Time: 1-min intervals.

The different cardiovascular and respiratory responses to hypoxia probably reflect ecological and behavioral differences among species. The sea raven, a sedentary fish, probably seldom if ever encounters low environmental oxygen conditions. Unlike those of trout and tench, the cardiovascular and respiratory control systems of sea ravens are insensitive to small changes in ambient PO_2 but probably come into play under severe conditions like those imposed during prey swallowing, which greatly reduces or stops gill irrigation for many minutes (Sutterlin and Saunders 1969).

EFFECTS OF SECTIONING CRANIAL NERVES ON EFFICIENCY OF BREATHING

Sea ravens, like tench (Hughes and Shelton 1962), still respond to hypoxia after bilateral section of cranial nerves IX and X. The most clear-cut effect of nerve section in sea ravens, a drastic reduction in dorsal aortic PO_2's, was probably owing to interruption of motor control of adductor and abductor muscles that normally position gill filaments so that the respiratory lamellae are efficiently irrigated by respiratory water. Pasztor and Kleerekoper (1962) emphasize the importance of these muscles in the active process of maintaining the gill curtain with respect to the respiratory flow. It is difficult to understand why there was no compensatory increase in breathing stimulated by low dorsal aortic PO_2's resulting from cranial nerve transection in view of the observed capacity to increase breathing during periods of reduced ambient PO_2.

A possible explanation for the observation that bilateral section of the glossopharyngeal nerves does not result in reduced dorsal aorta PO_2 is as follows: since the IXth nerve innervates the pseudobranch and the first gill arch and the Xth nerve supplies the remaining three gill arches, perhaps disruption of only about a quarter of the gill curtain was not sufficient to produce a marked drop in dorsal aortic PO_2. Moreover, there may be some mechanism to reduce blood flow through a damaged or otherwise ineffective gill.

The observation that breathing pressure waveforms in the opercular cavity were similar before and after bilateral section of cranial nerves IX

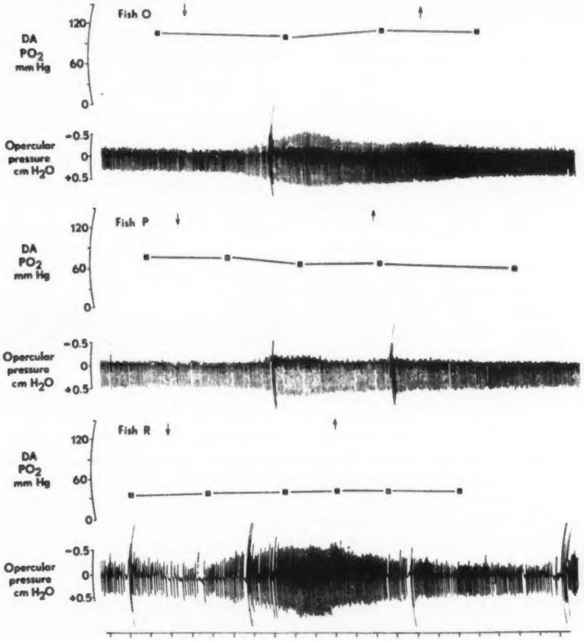

FIG. 8. Responses of three fish (O, P, R) to hypoxia during dorsal aortic perfusion with oxygenated blood. Arrows as in Fig. 6. Perfusion rate of pump same as in Fig. 7. Dorsal aortic PO_2 measured on blood after leaving artificial "gill." Time: 1-min intervals.

and X does not support the conclusions of Powers and Clark (1942) that these nerves are essential for maintenance of rhythmic breathing.

POSSIBLE RESPIRATORY CONTROL MECHANISMS

In spite of results of experiments wherein hypoxic responses were still evident after cranial nerves were sectioned, the possible existence of a peripheral receptor remains attractive. Should such a receptor system reside in elements innervated by nerves IX and X, the hypoxic response after section of these nerves could be owing entirely to receptors in the brain. Alternatively, the reflex could involve other than the glossopharyngeal and vagus nerves. The fifth and seventh cranial nerves, which supply motor control to the respiratory muscles and carry sensory information from the skin, lips, mouth, pharynx, and that part of the lateral line in the head, should be investigated. Such investigation would be difficult because section of these nerves results in cessation of breathing (Hughes and Shelton 1962). The olfactory system can probably be excluded because transection of the olfactory nerves during preliminary experiments in this investigation did not abolish the aforementioned responses to hypoxia.

Hypoxic responses resulting from perfusion of deoxygenated blood into the dorsal aorta whilst ambient PO_2 remained high may be interpreted as evidence for central oxygen receptor activity since the brain surely received deoxygenated blood. However, it is not known to what extent blood in the pseudobranch may have become oxygenated. It is likely that there was little change in the PO_2 of the perfused blood before it reached the pseudobranch, since blood reaches it via the afferent pseudobranchial artery, which leaves the anterior gill at its efferent end, thereby offering little or no opportunity for gas exchange. But it is possible that blood may have become oxygenated during passage through the pseudobranch, which is a large structure exposed to respiratory flow and not covered by a thick epithelium as in some teleosts.

Hypoxic responses resulting from perfusion of oxygenated blood into the dorsal aorta while

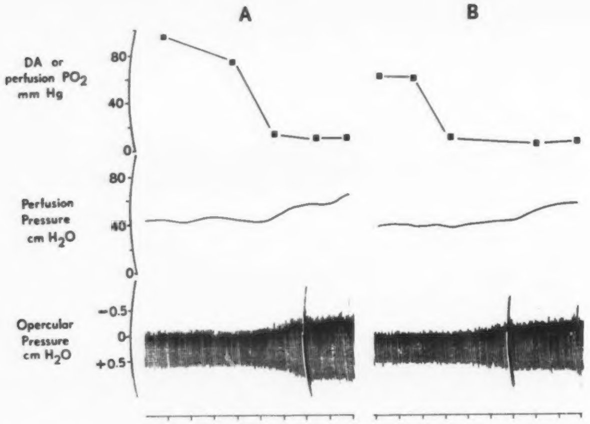

FIG. 9. Hypoxic responses of fish P during perfusion experiments. A, Breathing and blood pressure changes induced during ventral aortic perfusion with venous blood while subjecting the fish to environmental hypoxia. Top trace shows dorsal aortic PO_2 values; middle trace, perfusion pressure; bottom trace, breathing pressure in opercular cavity. B, Breathing and blood pressure changes during dorsal aortic perfusion with deoxygenated blood (N_2 substituted for air in the artificial gill). Top trace shows PO_2 in perfusion blood leaving artificial gill; middle trace, perfusion pressure; bottom trace, breathing pressure in opercular cavity. Time: 1-min intervals.

ambient PO_2 was low could not have originated from oxygen receptors in the brain, since its blood was well oxygenated, but must have originated peripherally. Since the ventral aorta was closed during dorsal aortic perfusion, blood within the gills would be stagnant and its PO_2 would tend to reach equilibrium with that of the ambient water. However, there would be some flow through the pseudobranch via part of the first gill arch and the afferent pseudobranchial artery as mentioned previously. So it is not known to what extent blood became deoxygenated during passage through the pseudobranch.

To what extent CO_2 and *p*H might be involved as chemical stimulants was not examined in our study. In most of our experiments the possible influence of CO_2 and *p*H can probably be excluded. It is likely that the artificial "gill" that was effective in oxygenating the venous blood was equally effective in removing CO_2 from the blood. During dorsal aortic perfusion with oxygenated blood at constant perfusion rates (Fig. 8), the responses to lowering ambient PO_2 probably did not include a component effect of CO_2 because PCO_2 in the perfusion blood would have remained constant and ambient PCO_2 would have been lowered, if changed at all. Responses to lowering ambient PO_2, while perfusing the ventral aorta at constant perfusion rate with unaltered venous blood (Fig. 7, 9A), were probably not complicated by effects of CO_2 on the brain or other central structure assuming the gills of the fish to have been as efficient in unloading CO_2 as in loading O_2, especially during periods of heavy breathing. The responses to dorsal aortic perfusion, at a constant rate, with blood equilibrated with N_2 in the "gill" (Fig. 9B) were probably not owing to CO_2 since the flow of N_2 through the "gill" should have been at least as effective in removing CO_2 from blood as the flow of air had been.

There is a possibility that chemical messengers released from the gills or other structures in contact with the blood or the ambient water effect hypoxic responses via receptors in the central nervous system. However, the existence of such a system involving the gills is unlikely in view of the dorsal aorta perfusion experiments in which there could be little or no flow of blood in either direction through the gills because the ventral aorta was occluded. Therefore, it is unlikely that chemical messengers released by the gills could have reached target organs in other parts of the body. However, there would be some blood flow through the pseudobranch and this structure should not be overlooked as a possibility for release of chemical substances.

The critical experiment remaining is to test various combinations of environmental hypoxia and normoxia during dorsal aortic perfusion with oxygenated and deoxygenated blood in a fish in which nerves IX and X have been cut. This should make it possible to establish whether there are central or peripheral, or both, sites of oxygen receptor activity. However, the stress imposed by the extensive operative procedures and by 2 successive days' attachment to the heart-gill apparatus is probably too severe. Certainly, several attempts failed during this investigation.

Possible Vasomotor Control Mechanism

The observed increase in dorsal and ventral aortic pressure during hypoxia is not caused by increased cardiac output alone because during ventral aorta perfusion with perfusion rate held constant, there was an increase in both perfusion and dorsal aortic pressure. Relatively constant differential pressure across the gills during hypoxia indicates little change in gill resistance compared with that in other peripheral blood pathways. This increase in peripheral resistance persisted after sectioning of nerves IX and X and was probably owing to a vasomotor reflex involving pathways of the autonomic nervous system other than those segments contained in nerves IX and X.

Acknowledgments

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Histological Comparisons of the Adrenal-Cortical Cells of Hatchery and Wild Landlocked Atlantic Salmon (*Salmo salar*)

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DAVIS, R. M., AND O. C. FENDERSON. 1971. Histological comparisons of the adrenal-cortical cells of hatchery and wild landlocked Atlantic salmon (*Salmo salar*). J. Fish. Res. Bd. Canada 28: 505–508.

No differences were found in mean diameters of adrenal-cortical cell nuclei between hatchery parr of landlocked Atlantic salmon (*Salmo salar*) killed immediately after removal from hatchery rearing pools and wild parr electrofished in two streams, and between hatchery and wild parr held for various periods in an outdoor stream aquarium simulating natural conditions. The similarities suggested that the wild and hatchery parr were similar in physiological response to environmental stress. In both hatchery and wild parr, the diameters differed at different times, indicating a downward trend from May to August in the one year of the study. No consistent relation was found between nucleus diameter and social behavior.

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THIS paper describes an attempt to determine whether environmental stress may be implicated in hitherto unexplained mortality of salmonids that commonly occurs in hatcheries or follows stocking in streams. The sensitivity of fish to alterations in the environment may have a considerable role in their growth, reproduction, and survival.

The effects of stress on fish can be shown by histological changes in adrenal-cortical tissues (Mahon et al. 1962; Rasquin and Rosenbloom 1954; Erickson 1967). These investigators have shown changes in adrenal-cortical tissues caused by environmental conditions, ranging from mild stimulation to exhaustion or deterioration. The diameters of adrenal-cortical cell nuclei (Olivereau 1965; Basu et al. 1965; Hanke and Chester-Jones 1966; Ball and Olivereau 1966) were used in this study as the measure of histological differences.

The present investigation was conducted to measure differences in the diameters of adrenal-cortical cell nuclei of hatchery parr of landlocked Atlantic salmon (*Salmo salar*) killed immediately upon removal from hatchery rearing pools and wild parr electrofished in two streams, and between hatchery and wild parr held for various periods in an outdoor stream aquarium simulating natural conditions. Parr from the stream aquarium were also used for studies on the comparative behavior of hatchery and wild salmon (O. C. Fenderson unpublished data). Survivors from each group studied were recovered by electrofishing and preserved for the present study.

Materials and Methods

Immature (age 1+) landlocked salmon of both sexes were used in this investigation. Hatchery-reared salmon were obtained from the Grand Lake Stream Fish-Cultural Station, and were progeny of wild parents from which spawn was collected in the fall of 1964 from the State of Maine. Wild salmon were obtained from Cold Stream and Mann Brook. Hatchery salmon were captured by dipnet from hatchery raceways, and wild fish with a pulsed d-c electrofishing unit. Fish remained in the electrical field only a few seconds and there was no evidence that their behavior or physical condition was adversely affected by electrofishing.

Details of the various study groups are given in Table 1. Hatchery parr dipped from hatchery rearing pools (groups 9, 11, and 13) and wild parr electrofished in two streams (groups 1, 3, 4, and 7) were killed immediately after capture for comparisons of cells from parr that had been reared in natural and artificial environments but had not been subjected to experimentation. Comparisons were also made between these two series of parr and both hatchery and wild parr that had been subjected to experimentation in the stream aquarium. The above study groups were collected at or near the same time parr were placed in the outdoor stream aquarium (described below) and again at the termination of a study period when aquarium fish were killed.

Six groups of parr, three hatchery (groups 2, 4, and 5) and three wild (groups 10, 12, and 14), were used for a study on the comparative behavior of hatchery and wild salmon in an outdoor stream aquarium simulating natural conditions. These fish were killed and used for the present study upon completion of behavior studies. Groups 2 and 10 were released in the aquarium on May 2, 1966, and were recovered by electrofishing after their behavior was observed for 31 days. Groups 4 and 12 were released on June 8, 1966, and observed for 14 days, and groups 5 and 14 were released on June 24, 1966, and observed for 41 days. These parr

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were subjected to natural fluctuations in stream flow, they fed on natural drift, sought shelter, competed for territories, and were attacked by kingfishers (*Magaceryle*) *alcyon*). The methods used for behavior studies and results can be obtained from O. C. Fenderson (unpublished data) but facts related to the present study are given below.

The stream aquarium was located at Jellison Brook and consisted of a wooden flume, 30.5 × 1.2 × 0.6 m deep, through which water from the brook was diverted. A 15.5-m long observation section was enclosed by screened barriers and divided into equal upstream and downstream sections by a center screen. Each section was provided with bottom materials and naturalized to provide cover, riffles, and pools. Fish activities were observed through windows along one side of the observation section. A black plastic canopy covering the observation section provided a darkened enclosure in which observers could move without unduly disturbing the fish.

All parr studied in the stream aquarium were transported to the Cobb Station and held 1 day in indoor rearing troughs for processing before they were transported to the stream aquarium. They were anesthetized with tricaine methanesulfonate, weighed, measured, and given marks for individual recognition either by clipping small portions of fins or by tatooing with titanium oxide.

Hatchery and wild parr were matched for size as nearly as possible, but hatchery parr usually averaged larger than wild parr. Hatchery and wild parr were released simultaneously in the aquarium, and either mixed in equal numbers in both sections of the observation area or separated by placing hatchery parr in one section and wild parr in the other.

Investigation of social behavior during these study periods afforded an opportunity to collect and compare adrenal-cortical cells from parr that had been subjected to the stresses imposed by social aggression and hierarchial relations. Three measures of social behavior were computed for each fish from data obtained in a systematic sequence of 5-min observations covering all fish in all areas of the aquarium.

Social status, a general measure of each parr's ability to dominate other individuals, was determined by summing three measurements: (1) the number of threats directed toward other fish divided by the number of threats received, (2) the number of other fish threatened divided by the number of fish from which threats were received, and (3) the number of threats given multiplied by the number of fish from which threats were received.

Frequency of aggression, a measure of the intensity with which each parr displayed aggressive behavior, was computed by dividing the total number of acts counted for each fish into total observation days.

The position of each parr in a hierarchy was determined from data on the interactions between individual parr inhabiting the same pool in the stream aquarium. Sufficient data were obtained for the 31- and 41-day groups for three pools in the lower section (labeled A, B, and C) and three pools in the upper section (D, E, and F). A parr was termed dominant to another parr if the number of aggressive acts directed toward that parr exceeded the number received from it.

HISTOLOGICAL TECHNIQUES

Parr were killed by a blow to the head and placed intact in Zenker's solution for fixation of head kidneys containing adrenal-cortical tissues. An incision was made in the abdomen of each fish to facilitate penetration of the fixative. Transverse sections were cut at $7\ \mu$ and stained with hematoxylin and eosin. A nucleus was treated as an ellipsoid, and its major and minor diameters measured in the plane of the section. These two diameters were averaged to give the mean diameter of an adrenal-cortical cell nucleus. The mean diameter of the adrenal-cortical cell nuclei for each parr was determined by measuring a total of 30 nuclei, 10 nuclei being taken at random from each of three areas.

STATISTICAL ANALYSIS

Statistical analysis of differences between nucleus diameters of parr from different groups was accomplished with Scheffé's (1953) test. The Kendall rank correlation coefficient (one-tailed probability of rejection) was used to test the relation between diameters of adrenal-cortical cell nuclei and social behavior (Table 2).

Results

No statistically significant differences were found in mean diameters of adrenal-cortical cell nuclei (Table 1) between hatchery parr killed immediately after removal from rearing pools and wild parr electrofished in streams (group 1 vs. 9 and 3 vs. 11), or between wild and hatchery parr at the end of the three study periods. The diameters were significantly smaller for hatchery parr held in the aquarium for 31, 14, or 41 days than for the pre-experimental parr (group 10 vs. 9, 12 vs. 11, and 14 vs. 13) and for wild parr held in the aquarium for 31 days than for the pre-experimental parr (group 2 vs. 1).

A pronounced seasonal trend toward decreasing diameters was evident in hatchery parr killed immediately after removal from hatchery rearing pools and in wild parr from streams, and also in both hatchery and wild parr held for various periods in the aquarium. Hatchery and wild parr examined in May had significantly larger nucleus diameters than those examined in August.

Low correlations were found between social status and mean diameter of adrenal-cortical cell nuclei ($r = 0.316$; $P = 0.043$) in the 41-day study group among wild salmon, and between nucleus diameter and position in a social hierarchy ($r = 0.264$; $P = 0.021$) in the 31-day group for hatchery and wild salmon combined (Table 2). Both probabilities indicated that the nucleus diameters tended to be largest among parr that were the most successful in dominating other parr. Among hatchery parr in the 41-day group, a negative correlation (r = -0.295) between nucleus diameter and social status was not significant (P = 0.059). The data for the two 31-day study groups were combined to increase sample size. Those on parr from the 41-day groups were not combined because hatchery and wild parr were held in separate compartments, and there was a significant difference in behavior;

TABLE 1. Mean diameters of adrenal-cortical cell nuclei of hatchery parr of landlocked Atlantic salmon killed immediately after removal from hatchery rearing pools and wild parr electrofished in two streams (controls), and of hatchery and wild parr held for various periods in an outdoor stream aquarium. Statistical comparisons are based on Scheffé's test (P = 0.05).

		Wild parr			Hatchery parr		
Exptl. conditions	Date of sampling (*1966*)	No. fish	Mean nuclear diam (μ)	Group no.	No. fish	Mean nuclear diam (μ)	Group no.
Controls[a]	May 5	17	6.5±0.06[b]	1	15	6.6±0.05	9
Stream aquarium 31 days[a]	June 6	8	5.8±0.06[c]	2	6	5.6±0.08	10[d]
Controls	June 8	11	5.8±0.05[c]	3	16	6.0±0.04	11[d]
Stream aquarium 14 days	June 21	5	5.6±0.07[c]	4	2	5.4±0.06	12[e]
Controls	June 22	–	–	–	7	**5.8±0.08	13[d]
Stream aquarium 41 days	Aug. 3	16	5.1±0.03[c]	5	15	**5.2±0.06	14[d,f]
Controls	Aug. 23	13	5.4±0.07[c]	6	–	–	–

[a] Taken from Cold Stream; other wild parr from Mann Brook.
[b] One standard deviation of the mean.
[c] Significantly different from value for group 1.
[d] Significantly different from value for group 9.
[e] Significantly different from value for group 11.
[f] Significantly different from value for group 13.

TABLE 2. Kendall rank correlation coefficients and associated probabilities for the relations between mean diameters of adrenal-cortical cell nuclei and social status, frequency of aggression, and position in hierarchy of salmon parr held in a stream aquarium for 31- and 41-day periods. Significant probabilities are shown by an asterisk (*).

	31 days			41 days					
		Wild and hatchery			Hatchery			Wild	
	No. fish	T[a]	P[b]	No. fish	T	P	No. fish	T	P
Nucleus diam vs. social status	14	−.264	.095	15	−.295	.059	15	.316	.043*
Nucleus diam vs. frequency of aggression	14	.103	.312	16	.200	.147	13	.026	.452
Nucleus diam vs. position in hierarchy:									
Pool A	8	−.143	.312	13	.192	.181	–	–	–
B	7	−.095	.382	15	.161	.203	–	–	–
C	6	−.200	.288	10	.494	.312	–	–	–
D	4	1.000	.021*	–	–	–	12	.133	.352
E	6	.066	.429	–	–	–	8	.222	.389
F	6	.000	.500	–	–	–	10	.227	.151

[a] Kendall tau.
[b] One-tailed probability of rejection.

hatchery parr were less shelter-oriented and more mobile than wild parr, and exhibited a higher frequency of agonistic behavior.

Discussion

The present study suggested that the hatchery-reared and wild parr were similar in physiological response to environmental stress. No differences were found in mean diameters of adrenal-cortical cell nuclei between hatchery parr killed immediately after removal from hatchery rearing pools and wild parr electrofished in streams or between hatchery and wild parr held for various periods in the outdoor stream aquarium.

No consistent relations between social behavior and the nucleus diameters were demonstrated. The meager evidence we obtained indicated that parr highest in social hierarchy and parr initiating the highest number of aggressive actions tended to have the largest nucleus diameters.

Our findings are opposite to that of Erickson (1967). He studied social hierarchy, territoriality, and stress reactions of pumpkinseed (*Lepomis gibbosus*) in 57-liter aquaria and found that the quantity of adrenal-cortical tissues of an individual fish was negatively correlated with the number of aggressive actions which that fish initiated, suggesting that the least aggressive fish was under the most severe stress. However, it should be emphasized that we used a different species of fish and the measure we used as an indicator of adrenal-cortical cell activity may not be comparable with that used by Erickson. Also, the low correlation between the nucleus diameter and social behavior in our study may have been attributable to the small number of fish represented in hierarchial social orders within pools of the stream aquarium, or to low sensitivity in our measurements of social behavior, or to both. However, the stream aquarium more closely approximated conditions that would be found in nature than did those used by Erickson. Salmon in the stream aquarium were much less confined than they would be in small laboratory aquaria, and ample cover provided for the salmon allowed them to hide and avoid continual stress. If diameters of adrenal-cortical cell nuclei are a true indicator of endocrine output and these cells are under the influence of social stresses, any gross influence of social stress on adrenal-cortical function in salmon parr would probably have been detected in this study.

The obvious decrease in the nucleus diameters from April through August among hatchery and wild parr may be a reflection of seasonal variation in activity of salmon. The feeding activity, social interactions, and general movement of these fish are markedly higher in spring than in summer and fall. Cell nucleus size may vary in relation to variation in hormone production and food consumption at different times of the year. The larger nucleus diameter found among hatchery parr taken immediately from the hatchery, as compared with similar groups of hatchery parr held for a time in the stream aquarium may also reflect changes in activity and food consumption. The metabolic activity of salmon from a hatchery pool where food is superabundant and population densities high might be expected to exhibit a high level of adrenal-cortical hormone output, as compared with that in parr living under more nearly normal conditions. On the other hand, wild parr in the stream aquarium may have existed under conditions not appreciably different from those in their native stream.

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Pigmentation of Brook Trout (*Salvelinus fontinalis*) by Feeding Dried Crustacean Waste

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SAITO, A., AND L. W. REGIER. 1971. Pigmentation of brook trout (*Salvelinus fontinalis*) by feeding dried crustacean waste. J. Fish. Res. Bd. Canada 28: 509–512.

Shrimp and crab wastes were fed to yearling brook trout for 12 weeks. Diets containing 20 and 30% shrimp waste, 20% crab waste, and 0.004% canthaxanthin were compared with a commercial trout diet. The carotenoid contents of the flesh and skin were highest with the canthaxanthin-fed fish, but the 20% shrimp-waste-fed fish had the most desirable color visually within the feeding period used.

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THE pigments of brown trout were found by Steven (1947) to be primarily astacene in the meat and lutein in the skin. He also investigated the deposition of carotenoids as the result of feeding β-carotene and other carotene-containing sources, and traced the transformation of the pigments in various organs (Steven 1948). Dried northern red shrimp waste has been reported to yield desirable color in cultured sea bream and colored carp (I. Ohnishi personal communication; Saito and Kondo 1967). Fresh raw shrimp waste, high-temperature air-dried and vacuum-dried shrimp waste meals have been used in Denmark. Visually, the fresh shrimp waste gave improved color whereas the high-temperature-dried waste showed no effect. The vacuum-dried waste also gave color and flavor improvement (Fishery Products Report 1968). A chemically synthesized carotenoid, canthaxanthin, has been fed to pink salmon, rainbow trout, and cutthroat trout, and significant pigmentation in the skin and flesh was observed (Deufel 1965; Brinchmann 1967; Schmidt and Baker 1968). The feeding of β-apocarotenal to rainbow trout did not give any accumulation of the carotenoid in the tissues but did cause an unattractive noncarotenoid color to appear in the flesh (Hirao et al. 1962).

The expansion of the crustacean-processing industry on the Atlantic coast of Canada has resulted in the production of large amounts of wastes. One possible use of some of these wastes is as a source of carotenoid pigments. The species chosen for the feeding studies on pigmentation was brook trout (*Salvelinus fontinalis*) because this species is being cultured commercially.

The purpose of this experiment was to determine the rate of incorporation of carotenoid in the crustacean waste into the muscle and skin of the brook trout.

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Materials and Methods

FISH

Eight-month-old brook trout were used. They were hatched and reared at the Liverpool Hatchery Station, N.S. The fish were brought to the Halifax Laboratory, stored in aquaria with aerated, flowing, brackish water. The controls and treated groups were kept in separate tanks with a maximum of 40 fish in one tank of 66 gal capacity. The temperature of water was kept at 6–9 C during the holding, and commercial trout food was fed at 2% of body weight per day for a 4-week period before the experimental carotenoid feeding was started.

FISH FEEDING

Normal diet — Silvercup trout food #1 (Murray Elevators, Utah: protein >50%, lipid >5%, fiber <7%, ash <15%, vitamin mix, and minerals) was used for control fish and as the mixing base for the carotenoid-containing diets. This trout food contained no measurable amount of carotenoids by spectrophotometric analysis of extracts.

Shrimp-waste diet — The pink shrimp (*Pandalus borealis*), caught off the Maine coast in February 1968, was received at the laboratory in a frozen state. After defrosting, the carapace and abdominal shell were carefully removed and dried in a vacuum oven at 20–30 mm Hg at a temperature below 50 C for 48 hr. The crude lipids were 10.7% of the sample. The pigments found in the extracts were astaxanthin 10.0 mg, unidentified ketocarotenoid 0.24 mg, astacene 0.91 mg, and noncarotenoidal pigments 1.08 mg per 100 g dry weight of sample (Saito unpublished data). Dried shrimp "waste" was ground with trout food to constitute either 20 or 30% of the mixture by weight. This was mixed with enough water to make a thick paste and then passed through a meat grinder. The spaghetti-shaped mix was dried in a stream of air at 25–30 C and broken into pellets. The diet was prepared every 2 weeks and stored in a refrigerator. This food was fed daily at 3% of the average fish weight.

Crab-waste diet — The snow crab (*Chinoecetes opilio*), caught off the New Brunswick coast in November 1968, were factory-cooked, frozen, and shipped to Halifax. They were cooked in boiling water another 7 min, the carapace and leg shell carefully removed and dried in a vacuum oven (28–30 mm Hg) at 55–60 C for 24 hr. Crude lipid was 1.22% of the dry shell. The pigments found were: a luteinlike carotenoid 0.01 mg, astaxanthin 0.47 mg, unidentified ketocarotenoid a trace, astacene 0.17 mg per 100 g dry weight of sample. No noncarotenoid pigments were found. The ground sample was mixed with normal trout food at 20% dry weight, and preparation of mixed pellets was the same as that of the shrimp-waste diet.

Canthaxanthin diet — Canthaxanthin, in the form of a 10% dispersion in a carrier of gelatine, sugar, and starch (Hoffmann-La Roche Ltd., Basle, Switzerland), was mixed with the trout food so that it contained 0.004% pigment. The pellet preparation and feeding rate were the same as in the shrimp-waste feeding experiment.

CAROTENOID ANALYSIS

Five fish were used for each sample representing one diet and time combination. Before feeding was started, analyses were conducted on control fish. After each 4-week period the fish were killed, the skin and meat were separated carefully and analysed for carotenoid content separately.

Meat — The meat of five fish was blended in a mixer. A portion was taken for moisture measurement. A 25-g wet sample was extracted with chloroform–methanol by the Bligh and Dyer method (1959). The chloroform was evaporated to dryness in vacuum under nitrogen. This crude lipid was dissolved in petroleum ether and the absorption spectrum was measured between 375 and 600 nm. When the curve had a peak at 460–470 nm, this absorbance was calculated as astaxanthin

TABLE 1. Muscle tissue analysis of the brook trout on experimental diets (averages for five fish).

Time (*weeks*)	Avg body length (*cm*)	Avg body wt (*g*)	Crude lipid (%)	Total carotenoid (*μg/100 g dry wt*)	Composition of major carotenoid fraction
			Control		
Initial	16	49	4.56	73	Mixture
4	16	48	4.80	52	Mixture
8	19	67	5.48	123	Lutein
12	20	88	7.59	139	Lutein
			20% crab-waste diet		
4	16	50	4.63	124	Mixture
8	17	59	5.72	132	Lutein
12	21	92	6.99	176	Lutein, astacene
			20% shrimp-waste diet		
4	16	67	5.23	510	Mixture
8	19	68	8.64	657	Astaxanthin
12	19	82	9.64	1430	Astacene
			30% shrimp-waste diet		
8	19	68	6.46	475	Astaxanthin, astacene
			0.004% canthaxanthin		
8	20	67	8.05	1980	Canthaxanthin, tunaxanthin (?), astacene

$E^{1\%}_{1cm}$ = 2200. This figure is used as the total carotenoid in this text. Further separation of the carotenoids was accomplished by column chromatography on alumina, and the components were identified by absorption spectra and thin-layer chromatography and by chemical characteristics of polarity (Saito unpublished data).

Skin — Skin parts, including all fins and the tail but none of the head, were used. A portion of the skin was reserved for moisture analysis. The remaining skin of five fish was cut into small pieces by scissors and extracted with hot acetone for 2 hr and kept at room temperature with stirring overnight. Acetone was removed under vacuum and the residue was transferred to petroleum ether. Analyses of the crude lipid, crude total carotenoid as lutein ($E^{1\%}_{1cm}$ = 2200 at 440 nm), and separation of carotenoids were the same as used on the meat samples.

Results and Discussion

FLESH COLOR

Fish length and weight, crude lipids, total crude carotenoids, and major carotenoid, are presented in Table 1.

During the 12-week experiment, the yearling trout appeared healthy and had less than 2% mortality. Neither food quotient nor food conversion rate was calculated because of the limited number of fish and the short term of feeding. No conclusion can be made as to which diet gave the best growth, but no marked growth-inhibiting effect was observed with the modified diets as compared with the control diet.

The moisture content of the flesh ranged from 72.3 to 77.4%. The content of crude lipid substantially increased with time, especially in the 20% shrimp-fed group, and in that on canthaxanthin diet, but the 20% crab-fed group remained at the same level as the control.

After 8 weeks on the experimental diets, the shrimp-fed fish had a pink-orange color that was subjectively salmonlike, whereas the canthaxanthin-fed fish had a deep orange color that seemed unusual and excessively bright for fresh trout. This is in contrast to the observations of Schmidt and Baker (1969). However, they were interested in pigment levels that on heat sterilization would be near those of the more highly pigmented Pacific salmon. The color intensity appeared correlated with the crude carotenoid content.

The detectable carotenoids in the flesh became more varied when the feeding was prolonged and more carotenoids were deposited. The major component, lutein, in the earlier stage was supplanted by astaxanthin and astacene. Since lutein has an alpha-carotene-based structure, it is unlikely that it would be converted to the beta-carotene type such as astaxanthin.

SKIN COLOR

The results of the analyses of the skin samples are shown in Table 2.

The color of the skin was significantly affected by the deposition of carotenoids after 8 weeks of feeding on the shrimp-fortified diet. The skin color became brighter and had more intense hues all over. The pink spots on the skin surface became markedly deeper, and the belly fins and anal fins showed a deep orange color. A small amount of this coloration was observed in the control group fed the commercial diet. The group fed 30% shrimp diet after 8 weeks was similar to the group fed the 20% shrimp diet after 12 weeks. The canthaxanthin-fed group had a high content of carotenoids after 8 weeks, but the overall skin

TABLE 2. Skin carotenoid in the brook trout fed diets containing carotenoids (averages for five fish).

Time (*weeks*)	Total carotenoid (*μg/100 g dry wt*)	Major carotenoid	Ratio to control
	Control		
Initial	214	Lutein	1.00
4	200		
8	1230		
12	1250		
	20% crab-waste diet		
4	220	Not identified	1.10
8	1260	Lutein	1.03
12	1790	Lutein	1.40
	20% shrimp-waste diet		
4	332	Not identified	1.66
8	2140	Lutein	1.74
12	3400	Lutein, astaxanthin	2.72
	30% shrimp-waste diet		
8	3700	Lutein, tunaxanthin (?), astaxanthin	3.02
	0.004% canthaxanthin diet		
8	6480	Echinenone, lutein, canthaxanthin, astacene	5.20

color appeared to be the same as the shrimp-fed group. The crab-fed group did not show a rapid deposition of carotenoids.

SENSORY EVALUATION

Three samples of fish fed on the diets — control, 20% crab diet, and 20% shrimp diet — were served to a panel for evaluation. The trout were filleted, wrapped in aluminum foil, and baked in a 350 F oven for 8–10 min. The cooked samples were served to a 17-member panel of laboratory employees experienced in fish testing. The panel members were asked to rank the three samples with respect to skin appearance, flesh appearance, flesh odor, and taste. The canthaxanthin-fed fish had such a deep color in the meat that they were excluded from the taste and odor tests. The 20% shrimp-waste-fed fish were ranked first by the panel on all four attributes. The crab-fed fish were ranked second for appearance and the control fish were ranked second for taste and odor.

These studies have shown that the carotenoid compounds in dried shrimp waste can be incorporated into the flesh and skin of brook trout within a period of 8–12 weeks. These compounds contribute a much more desirable appearance to the skin and flesh of the fish than is found in fish fed on a diet nearly devoid of the materials. The crab waste was found to have only a slight effect on the pigmentation of the trout.

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A Hypothesis of Alternation of Age of Return in Successive Generations of Skeena River Sockeye Salmon (*Oncorhynchus nerka*)

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Bilton, H. T. 1971. A hypothesis of alternation of age of return in successive generations of Skeena River sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 28: 513–516.

Egg weight of Scully Creek and Lower Babine River sockeye salmon (*Oncorhynchus nerka*) was positively correlated with length and, usually, age of the female parent. Egg weight was positively correlated with initial size and subsequent growth of juveniles, at least up to 3 months of age. Examination of scales indicated juvenile growth in the lake and ocean was inversely related to age at maturity. Hence it was hypothesized that the larger age 1.3 sockeye spawners tend to produce progeny that mature as smaller age 1.2 fish, which in turn give rise to progeny that mature as larger age 1.3 fish, and so on; an alternation in age of return of successive generations occurs.

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In North America, sockeye salmon (*Oncorhynchus nerka*) may mature in their 3rd–8th year of life (Mosher 1963). They typically spend 1 or 2 years in lakes before migrating to sea. Sockeye in most of the major British Columbia stocks migrate to sea early in their 2nd year and the age at which they mature varies widely from brood year to brood year (Fraser River sockeye are an exception, with most maturing in their 4th year of life). Fish in their 4th and 5th years that migrated to sea in their 2nd year (age 1.2 and 1.3 fish[1]) have made up most of the adult returns to the Skeena River and Rivers and Smith inlets. Although the average proportions of age 1.2 and 1.3 Skeena River sockeye that returned from each brood year for the past 50 years have been about equal (Bilton et al. MS 1967). Some marked departures have occurred with 1.2's forming as few as 21% and as much as 81% of the total return. Such wide annual fluctuations in the age at maturity have a profound influence on the number of fish returning in any one year, and make difficult the task of assessing the production of adult sockeye of specific brood years. There is need to understand the factors that influence the age of maturity.

The purpose of the studies reported here was to examine the extent of maternal influences (female size or age, or both, and egg size) on age at maturity of Skeena River sockeye salmon. These influences and several other correlated phenomena, lead to a hypothesis of alternation in age of return in successive generations, i.e., age 1.2 female sockeye tend to produce age 1.3 offspring, of which the females in turn tend to produce age 1.2 offspring. A detailed report of this study has been published by the author (Bilton MS 1970). The purpose of the present paper is to summarize the findings given there.

Materials and Methods

In September 1964, at Scully Creek, a tributary to Lakelse Lake in the Skeena River drainage, eggs were taken from 19 female sockeye salmon of known age and size and fertilized with sperm of 15 males of known age and size (Tables 1 and 2).

In January 1965, individual weights of 50 "eyed" eggs from each female were obtained.

In October 1965, eggs were taken from 89 age 1.2 sockeye from the Babine River, at the outlet of Nilkitkwa Lake, fertilized, and after water hardening, lots of 50 eggs from each female were weighed.

In September 1966, eggs were taken from 43 age 1.3 sockeye from the Babine River. In this instance eggs were not fertilized, were allowed to water harden, and 100-egg lots were weighed from each female. Statistical tests indicated the weights of fertilized and unfertilized

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[1] In the present paper the European system of age designation is used. Here, the number of annual rings formed is indicated. For example, a "1.2" is a fish with one annual ring that was formed on its scales while it was in fresh water, and with two annual rings that were formed while it was in the ocean. A 1.2 by this system is the same as a 4_2 in the Gilbert system, where age refers to the year of life dating from time of egg deposition.

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eggs from the Babine River were not significantly different (F = 0.150; df 1, 8; $P > 0.05$).

The eggs from 16 of the 19 Scully Creek females mentioned earlier were allowed to hatch and the progeny were reared in separate tanks until October 1965. During this period the conditions in each tank were kept as similar as possible. Temperatures and the number of fish held in each tank were approximately the same, and all fish were provided with an ad libitum ration. Samples of these fish were weighed and measured 1, 2, 3, and 9 months after hatching.

Scales of 6513 age 1.2 and 1.3 Skeena sockeye sampled from the catch and escapement of 4 brood years (1952–55) were examined under a microprojector at a magnification of 100 diameters. The number of circuli in the freshwater year band were counted, or the width of year band was measured, or both.

TABLE 1. Numbers of age-class crosses completed on Scully Creek sockeye salmon, 1964.

Female age	Male age 1.1	1.2	1.3
1.2	2	3	3
1.3	3	0	5

Results

Within a brood year age 1.3 adult female sockeye tended to be larger than age 1.2 adult female sockeye (Babine sockeye; Table 2).

Analysis of variance of the Scully Creek and Babine River sockeye indicated significant positive relations between egg weight and length of parent female (probably age too, as the older fish tended to be larger than the younger fish) (Table 2). For Scully Creek, F = 31, $P < 0.005$, df 1, 17; Babine River 1.2's, F = 22.69, $P = 26 < 0.001$, df 1, 88; and 1.3's, F = 4.61, $P < 0.05$, df 1, 42.

Measurements of fish from the 16 crosses of age 1.2 and 1.3 sockeye (Table 3) indicated that the mean length and weight of young fish 3 months after hatching were positively, and significantly, correlated with both egg weight and the length of female parent (r values = 0.601–0.960). There was no significant correlation between length of fry at 3 months and length of the male parent in each cross (r values = 0.000–0.033). Comparisons of mean lengths and weights of 9-month fingerlings of 11 crosses (only 11 crosses had survived up to this time) with length of female or mean egg weight produced smaller, but still positive, correlation coefficients (female length vs. size $r = 0.585$, 0.595, $P < 0.05$, < 0.10; and egg weight vs. size; $r = 0.492$, 0.473, $P < 0.10$).

TABLE 2. Mean egg weight for sockeye salmon, by length and age, from Scully Creek in 1964 and Lower Babine River in 1965 and 1966.

	Scully Creek			Lower Babine River		
Hypural length of female (*cm*)	Age	Mean egg wt (*g*)	No. females sampled	Age	Mean egg wt (*g*)	No. females sampled
39	1.2	.1252	2	–	–	–
40	1.2	.1045	1	1.2	.1246	1
41	1.2	.1152	3	–	–	–
42	1.2	.1408	2	1.2	.1317	11
43	1.2	.1265	2	1.2	.1349	12
44	–	–	–	1.2	.1492	15
45	1.3	.1447	1	1.2	.1477	24
	–	–	–	1.3	.1417	2
46	1.3	.1402	1	1.2	.1490	16
47	–	–	–	1.2	.1500	9
48	1.3	.1552	2	1.2	.1620	2
	–	–	–	1.3	.1552	3
49	1.3	.1505	2	1.3	.1546	4
50	1.3	.1383	1	1.3	.1704	6
51	1.3	.1615	2	1.3	.1655	12
52	–	–	–	1.3	.1737	5
53	–	–	–	1.3	.1591	6
54	–	–	–	1.3	.1754	4

TABLE 3. Correlations between female length, egg weight, and weight and length of resulting 3-month fry and 9-month fingerlings, for Scully Creek sockeye, 1964.

	Female length		Egg wt	
Correlation	r	P[a]	r	P[a]
Egg wt	+0.791	<0.01	...	...
Fry length (3 months)	+0.660	<0.01	+0.601	>0.01; <0.02
Fry wt (3 months)	+0.711	<0.01	+0.960	<0.001
Fingerling length (9 months)	+0.585	>0.05; <0.10	+0.492	>0.10
Fingerling wt (9 months)	+0.595	>0.05; <0.10	+0.473	>0.10

[a] P = probability of chance occurrence.

Comparison of age 1.2 and 1.3 sockeye from the 1952–55 brood years (Table 4) indicated age 1.2 fish had significantly more freshwater circuli than age 1.3 sockeye from the same brood. Because circulus counts and year band widths on scales from migrant yearling Babine Lake sockeye were positively correlated with fish length (Bilton MS 1970), the data suggest that age 1.3 adults tended to originate from smaller yearlings than did age 1.2 adults.

TABLE 4. Weighted mean number of freshwater circuli on the scales of age 1.2 and 1.3 Skeena River sockeye, brood years 1952–55.

	Age		
	1.2		1.3
	1952		
$\bar{x}$	13.70		11.60
SD[a]	2.57		1.44
N	732		872
t		21.2[b]	
% <12 circuli	12.6		50.2
	1953		
$\bar{x}$	12.75		11.52
SD	1.23		1.53
N	1305		1100
t		18.2	
% <12 circuli	17.6		56.1
	1954		
$\bar{x}$	13.16		11.48
SD	1.33		1.47
N	948		764
t		20.7	
% <12 circuli	10.9		53.9
	1955		
$\bar{x}$	13.09		12.79
SD	2.38		3.56
N	405		387
t		1.74	
% <12 circuli	12.8		23.8

[a] SD, standard deviation.

[b] Underlined value of t indicates significance at the 5% level.

Conclusions

Age 1.3 adult female sockeye tend to be larger than age 1.2 adult female sockeye. Because larger females tend to produce larger eggs, eggs of age 1.3 female sockeye tend to be larger than those of 1.2 female sockeye. At least up to 3 months after hatching and probably longer, young fish hatched from larger eggs tend to be larger than those hatched from smaller eggs.

Assuming that this superiority in size continued through the full first year (a point on which corroborating data are not yet available), the progeny of age 1.3 females would tend to migrate to sea as larger smolts than would the progeny of age 1.2 females. As inferred from relations between 1st-year circulus counts of scales and size of seaward migrants, small smolts tend to return more often as age 1.3 fish than do large smolts. Thus the progeny of the large 1.3 female sockeye producing large eggs resulting in large seaward migrants would tend to mature as smaller age 1.2 fish that would, in turn, tend to produce smaller progeny returning as age 1.3 and so on. Thus there would tend to be alternation in age of return between successive generations.

Acknowledgments

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First Records of Vibriosis in Pacific Salmon Cultured in Canada, and Taxonomic Status of the Responsible Bacterium, *Vibrio anguillarum*

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EVELYN, T. P. T. 1971. First records of vibriosis in Pacific salmon cultured in Canada, and taxonomic status of the responsible bacterium, *Vibrio anguillarum.* J. Fish. Res. Bd. Canada 28: 517–525.

First proven outbreaks of piscine vibriosis in Canada are recorded. The disease occurred in four species of Pacific salmon that were being cultured in sea water at facilities in Nanaimo and West Vancouver, B.C., and both the disease and the causative bacterium (identified as *Vibrio anguillarum*) are described.

A brief review of the literature on *Vibrio* fish pathogens suggests that most of these organisms might reasonably be assigned to the species *V. anguillarum*, and a tentative archtype for the species is described. Before the species is formally recognized, however, a large-scale comparative study is needed to provide the uniform information that is now lacking for individual strains of this species and to determine whether in fact it can be distinguished from other heterotrophic marine vibrios, many of which are commensal with marine animals.

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VIBRIO infections of fish have been widely reported (Anderson and Conroy 1970), but reports thus far from North America have come only from the United States (Rucker et al. 1953; Ross et al. 1968; Cisar and Fryer 1969). Recently, the disease was diagnosed in cultured Pacific salmon held at two locations on the west coast of Canada. The primary purpose of this paper is to describe the disease and the strains of the causative bacterium (*Vibrio anguillarum*) involved in these epizootics. Because, however, *V. anguillarum* has never been formally recognized in any of the editions of Bergey's *Manual of Determinative Bacteriology*, and because much uncertainty still exists as to criteria defining the organism (Egusa 1969), the taxonomic status of the species is also discussed.

The Disease

Four species of salmon were affected. These were being held in separate tanks in running sea water. At the time of the disease outbreaks, water temperatures approximated the annual maxima normally expected at the facilities involved: 15 C at the Nanaimo Biological Station, and 12 C at the Pacific Environment Institute, Fisheries Research Board of Canada, West Vancouver.

At the Nanaimo Station, the disease was first observed on July 22, 1968, in a stock of underyearling chum (*Oncorhynchus keta*) salmon. Three days later, it was observed in a stock of 2-year-old sockeye (*O. nerka*) salmon. In the chum stock, mortalities were so sudden and severe (almost 50% of the 650 fish were dead within 4 days) that attempts at chemotherapy proved too late to be of any practical value. With the sockeye stock, perhaps because of the larger size of the fish and the fact that terramycin treatment was immediately initiated, mortalities were less severe and the disease was controlled.

In West Vancouver where lower water temperatures prevailed, the disease was more protracted. Mortalities in yearling pink (*O. gorbuscha*) and chinook (*O. tschawytscha*) salmon extended over a 2-month period (August and September 1969). These stocks, which had never been treated for the disease, were deliberately killed in late September when the mortality rates suddenly increased.

Externally, dead and dying fish frequently exhibited redness at the base of the paired fins, vent, and gill isthmus. These symptoms were usually accompanied by large diffuse haemorrhages on the body surface and in the musculature. Internally the symptoms were less striking: the intestine was often inflamed and petechiae were sometimes present on the viscera. Death usually supervened before open external lesions or necrotic changes of the internal organs were evident.

Gram-stained preparations of blood, kidney, liver, and spleen revealed large numbers of gram-negative, straight and slightly curved rods (in specimens that had been frozen, many of the rods

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appeared to have "exploded"). Heart blood from moribund fish contained vast numbers of actively motile rods. In a few instances, the rods occurred in short chains and under these conditions, the organisms assumed a compound S-conformation.

Materials and Methods

CULTURES

Four strains of bacteria representing cultures isolated from the four affected species of salmon were studied. Strains V1 and V2 were isolated during the 1968 epizootic from the heart blood of moribund chum and sockeye salmon, respectively. Strains V3 and V4 were isolated during the 1969 disease outbreak from the kidneys of diseased pink and chinook salmon, respectively. The latter fish samples were sent, frozen, to this laboratory for examination.

Tissues for culture were obtained aseptically from fish that had not been treated with antibiotics. Isolations were made at 22 C on plates of TYE agar (tryptone 1.0%; yeast extract 0.5%; NaCl 0.5%; agar 1.5%) and judging from cell and colony morphology, pure cultures were obtained from all specimens.

CHARACTERIZATION TESTS

Tests were performed on cultures grown at 25 C in media prepared mainly with Difco products or in media prepared commercially by Difco (the latter are designated by the prefix "Bacto"). Ingredients not obtained from Difco (glycogen, organic acids, amino acids, inorganic salts, Tween compounds[1]) were of reagent grade. Citrated human blood, used for detecting haemolysis, was kindly supplied by the Nanaimo Regional General Hospital.

Filter-sterilized media, or media prepared with filter-sterilized ingredients (urea, gluconate, and carbohydrate broths except for starch broth) were incubated at 25 C for several days before inoculation to check for sterility.

Log-phase cells from TYE agar or broth routinely served as inocula but where the carry-over of nutrients might have led to falsely positive results (citrate, malonate, and tartrate broths), a loopful of cells from a TYE agar slant was suspended in 10 ml of sterile 1% saline to provide a cell suspension with barely detectable turbidity. Media were immediately inoculated with this material (0.02 ml of cell suspension/5 ml test system).

Appropriate controls were always employed. These included the use of uninoculated media, the use of inoculated media with the test substrate omitted, and in certain instances, the use of stock bacterial cultures (*Aeromonas liquefaciens* and *A. salmonicida*) with known properties.

Tests for the following reactions were conducted at 2, 4, 7, and 14 days: methyl red, acetoin, 2,3-butanediol, phenylalanine deaminase, and gluconate oxidation. All other tests were checked for 21 days unless otherwise specified. Where negative results were obtained in the media described, reactions were retested in the media supplemented with NaCl (3% final concentration).

1. Tests for acid and gas production from "carbohydrates" were conducted in Bacto purple base broth containing 1% test substrate.

2. The oxidative or fermentative nature of the cultures was determined with glucose in Bacto OF basal medium according to the method of Hugh and Leifson (1953).

3. Indole production was determined at 48 hr with Kovacs' reagent on cultures grown in tryptone broth: tryptone 1.0%; NaCl 0.5%.

4. Acetoin (detected with Barritt's reagent), methyl red, and 2,3-butanediol reactions were determined on cultures grown in Bacto MR-VP medium.

5. The production of nitrite from nitrate was determined on day-old cultures grown in Bacto tryptic-nitrate medium. All reagents used in tests 3–5 are described in the Difco Manual[2] except that for 2,3-butanediol (see Bullock 1961).

6. Proteolysis was determined in Bacto nutrient gelatin and in Bacto litmus milk.

7. Haemolysis was determined on streaked plates of Bacto tryptose blood agar base containing 0.5% (v/v) human blood.

8. Lipolytic activity was determined according to the method of Sierra (1957) with 1% (v/v) Tween 40 and Tween 80, respectively, as test substrates. Tests 7 and 8 were recorded at 3 days.

9 and 10. Tests for hydrogen sulphide production and urease activity were performed in stab-inoculated Bacto SIM medium and in Bacto urea broth, respectively.

11. Cellulase activity was determined in the following broth: tryptone 0.25%; yeast extract 0.1%; NaCl 0.5%. Whatman No. 1 paper strips immersed in the broth were observed for 30 days for evidence of disintegration.

12. Tests for decarboxylase activity were conducted at *p*H 6.0 and under mineral oil in Bacto decarboxylase base, Moeller, with 1% arginine·HCl, ornithine·HCl, and lysine, respectively (Difco Supplementary Literature, p. 68).[3]

13. Phenylalanine deaminase activity was determined on slants of Bacto phenylalanine agar using the reagent described in Difco Supplementary Literature (p. 280).

14. The oxidation of potassium gluconate was determined according to the method of Haynes (1951) except that the medium contained 0.5% NaCl and the flasks were shaken by hand once daily. Benedict's qualitative reagent was used to test for reducing substances.

15. The ability of the cultures to utilize citrate, malonate, and tartrate for growth (each supplied separately as the sodium salt at a level equivalent to 0.5% of the free acid) was tested at *p*H 6.9 in a modified Koser's

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[1] Polyoxyethylene sorbitan ester of oleic acid (Tween 80) or palmitic acid (Tween 40).

[2] Difco Laboratories Inc., Difco Manual (9th ed.), Detroit, Michigan.

[3] Difco Laboratories, 1968. Difco Supplementary Literature, Detroit, Michigan.

salt solution ($NaNH_4HPO_4 \cdot 4H_2O$ 1.5%; KH_2PO_4 1.0%; $MgSO_4 \cdot 7H_2O$ 0.02%; NaCl 3.0%) and in the salt solution enriched with 0.5% vitamin-free casitone. Controls consisted of the inoculated basal media and the inoculated basal media to which a utilizable carbon source (0.5% glucose) had been added. Tubes were observed daily for growth and turbidities were read spectrophotometrically at 21 days.

16 and 17. Tests for the oxidase (Kovacs 1956; Ewing 1962) and catalase (Davis and Park 1962) reactions used 48-hr growth obtained from TYE agar.

18. Drug sensitivity tests were performed by placing antibiotic discs on the surface of uniformly seeded TYE agar. Zones of growth inhibition were recorded at 24 hr. The following antibiotics were tested: penicillin (2 and 10 units), novobiocin, chloromycetin, aureomycin, terramycin (5 and 30 μg of each), and the vibriostatic agent 0/129 (2,4-diamino-6,7-di-isopropyl pteridine[4]) (20 μg).

VIABLE CELL DETERMINATIONS

Viable cells were quantified by diluting a sample of the cell suspension in TYE broth (10-fold serial dilutions), incubating the broth dilution tubes at 25 C for 5 days, and noting the highest dilution yielding growth. Occasionally, the highest dilution tube showing typical growth was preceded by one without growth. In such instances, the tube without growth was taken as the end point as plate counts conducted in parallel had established that this might be expected (with the conditions used, the transfer of a single viable cell was all that was required). To avoid death due to thermal shock, dilutions were performed in broth adjusted to the temperature of the cell suspension and the broth dilution tubes were then allowed to rise to 25 C in air.

Results

CELL MORPHOLOGY

Young (20-hr) cells of all four strains were gram-negative, nonacid-fast rods. They occurred both straight and slightly curved, were vigorously motile, and when stained by the Blenden and Goldberg (1965) method, were shown to possess a single polar flagellum (the occasional cell bore a single flagellum at each pole, but such cells may have been dividing) (Fig. 1). Cell size varied according to source and stain: cells in Wright-stained films of heart blood averaged $2.7 \times 1.0\ \mu$, but young Gram-stained cells from TYE agar averaged only $1.4 \times 0.5\ \mu$. In older (5- to 10-day) TYE agar or broth cultures, coccoid, spheroplast-like forms predominated. In contrast to the rod forms that were also present (these occurred straight or curved, singly or in very long chains), the coccoid forms stained faintly and unevenly, possessed indistinct outlines, and were nonmotile. Spores and capsules were never observed.

GROWTH CHARACTERISTICS

Growth of the four strains was not particularly striking in appearance. In TYE broth, a uniform turbidity and fragile pellicle rapidly developed but in a few days most of the growth had settled to produce a viscous sediment. On TYE agar, 48-hr colonies were circular, 1–2 mm in diameter, shiny, convex, butyrous, grey to faint yellowish-grey, and translucent, but on this and other suitable growth media tested (Bacto furunculosis agar, Bacto tryptic soy agar, Bacto nutrient agar prepared with sea water or 3% NaCl), noteworthy features like diffusing pigments, agar digestion, fluorescence, and luminescence were never observed.

EFFECTS OF SODIUM CHLORIDE

The strains required NaCl for growth and survival. Unlike stock cultures of two freshwater pathogens (*A. liquefaciens* and *A. salmonicida*) tested in parallel, they failed to grow in Bacto nutrient broth unless NaCl was added to the medium. They grew with levels of added NaCl ranging from 0.1% (lowest level tested) to 8.0% (highest level permitting growth), but at these extremes growth was delayed for a day or two. Fastest growth occurred in a tryptone–yeast extract solution (or in Bacto nutrient broth) with 1% added NaCl (Fig. 2*a*) but maximum cell yields were always obtained with higher levels (about 3%) of added NaCl (Fig. 2*b*).

Perhaps because even low levels of naturally present salt sufficed for growth (in Fig. 2*a* measurable growth occurred in 20 hr in TYE broth prepared without salt), the importance of salt to these organisms was at first overlooked. In fact, the first clue to a possible salt requirement came when cells suspended in distilled water (preparatory to flagella staining) were observed to become instantly nonmotile. This loss in motility was correlated with a loss in viability. In distilled water, loss in viability was so rapid that estimates of the initial viable cell population could only be obtained by reference to a system (1% NaCl) in which the cells were more stable (Fig. 3). However, even though in 1% NaCl the viable cell population remained constant for 2 hr (Fig. 3), by 24 hr, only 0.01% of the cells were still viable. Survival could be prolonged if organic matter (0.1% peptone) was also present (Table 1). Peptone alone was about as inefficient in extending survival as NaCl alone, but when used together, the beneficial

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[4] Supplied by Allen and Hansburys Ltd., Ware, Herts, England.

TABLE 1. Survival of strain V1 cells in various suspending media at 0–3 C.

Suspending medium	Viable cells/0.45 ml of suspending medium at indicated time (*days*)													
	0	1	2	3	4	5	6	7	14	21	27	36	42	50
Distilled water	10^5	0												
Distilled water + P[a]	10^5	10^2	10^1	0										
1% NaCl	10^7	10^2	10^1	0										
1% NaCl + P	10^6	10^6	10^6	10^5	10^6	10^6	10^6	10^6	10^5	10^4	10^5	10^2	<10	0
3% NaCl	10^6	10^1	0											
3% NaCl + P	10^6	10^6	10^6	10^5	10^6	10^7	10^5	10^5	10^4	10^4	10^3	0		
Sea water[b]	10^7	10^6	10^4	10^3	10^4	10^3	10^3	10^3	10^1	0				
Sea water + P	10^6	10^5	10^6	10^7	10^5	10^7	10^7	10^6	10^7	10^8	10^9	10^8	10^8	10^7

[a] P = peptone, 0.1%.

[b] Sea water (0.45 μ Millipore filtered and autoclaved 10 min) from Departure Bay, B.C. Salt content 3.0% when dried to constant weight at 110 C. Procedures are given in Fig. 3 and in Methods. Between samplings, cell suspensions were incubated static.

effect on survival was pronounced. This effect was most pronounced with filtered, autoclaved sea water in which an already appreciable survival of between 2 and 3 weeks was extended to indefinite survival (with perhaps some limited growth) by including peptone in the system.

EFFECTS OF TEMPERATURE AND pH

For strain V1 at 20 hr, growth in TYE broth was fastest at 20–25 C and at *p*H 6.5–7.5 (Fig. 4*a* and *b*). These results were also true of the other three strains except that their optimum *p*H ranges were somewhat broader (*p*H 6.5–8.0 for strain V2; *p*H 6.0–7.5 for strains V3 and V4).

The strains all grew at temperatures ranging from 1 or 2 C (light growth in 14 days) to 35 C (detectable growth in 20 hr). They failed to grow at 37 C at which temperature they were killed within a few days.

BIOCHEMICAL PROPERTIES

Biochemically, the strains were identical.

Acid, but no gas, was produced from the following substrates: ribose, glucose, galactose, fructose, mannose, sucrose, maltose, trehalose, cellobiose, starch, dextrin, glycogen, mannitol, sorbitol, glycerol (delayed 1–2 days), and inositol (delayed 3–5 days, and only with 3% NaCl). The following substrates were not attacked even when the basal medium contained 3% NaCl: arabinose, xylose, rhamnose, lactose, melibiose, raffinose, melizitose, adonitol, dulcitol, inulin, and salicin.

According to the Hugh and Leifson (1953) test, the strains were anaerogenic fermenters since they produced acid, but no gas, anaerobically from glucose.

Both acetoin and 2,3-butanediol were rapidly produced from glucose. Based on the intensities of the colour reactions, the strains produced more 2,3-butanediol and less acetoin than *A. liquefaciens*, which was also positive for these reactions. The methyl red reaction was negative whether or not 3% NaCl was present.

The strains produced indole (less strongly than *A. liquefaciens*), reduced nitrates to nitrites, hydrolyzed both Tween 40 and Tween 80, and were β-haemolytic on human blood. Gelatin was liquefied (the time for 100% liquefaction was reduced from 7 to 3 days when 3% NaCl was present). In litmus milk, most of the casein was digested in 21 days. Digestion was accompanied by the formation of a soft coagulum, a gradual reduction of the litmus, and a slight decrease in *p*H (from *p*H 6.4 to 6.1).

Arginine was rapidly attacked but only when 3% NaCl was present. Whether the attack was due to decarboxylase or dihydrolase activity was not determined. Tests for the decarboxylation of ornithine and lysine, the deamination of phenylalanine, the hydrolysis of urea, the production of hydrogen sulphide, and the decomposition of cellulose were all negative whether or not 3% NaCl was present.

Gluconic acid was attacked with the production of reducing substance(s). Other products were acetoin and 2,3-butanediol. Consistent results were obtained only when 3% NaCl was present and the accumulation of reducing materials occurred after a lag period of 2–4 days.

The strains failed to grow in Koser's salt solution (with 3% NaCl) when citrate, malonate, or tartrate

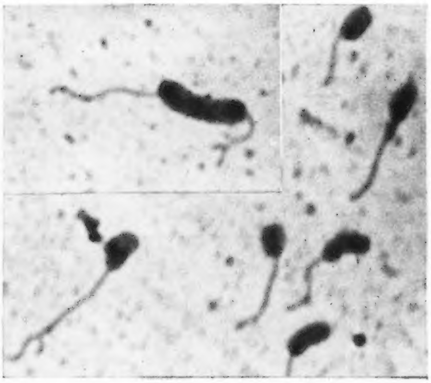

FIG. 1. Flagellar arrangement of strain V1 cells. Strain V1 cells from a 20-hr TYE agar culture were suspended in distilled water containing 1% formaldehyde to give a light suspension. Air-dried films of the suspension were stained according to Blenden and Goldberg (1965). ×3100.

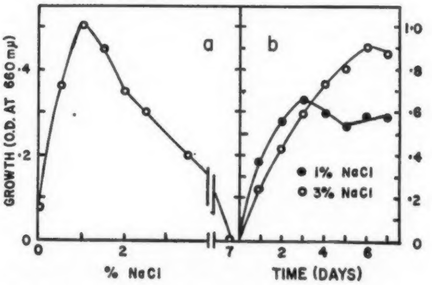

FIG. 2. Effects of NaCl concentration on yield of strain V1 cells at 20 hr and at maximum stationary growth phase. Effects of added NaCl on growth were determined at 25 C in 1.0% tryptone–0.5% yeast extract broth. Effects at 20 hr (*a*) were determined in tubes; effects at maximum stationary growth phase (*b*) were determined in flasks with 1 and 3% added NaCl. Flasks were well shaken before each sample was withdrawn and samples were frozen and read as a batch after appropriate dilution at the end of the experiment. Values plotted represent turbidity before dilution.

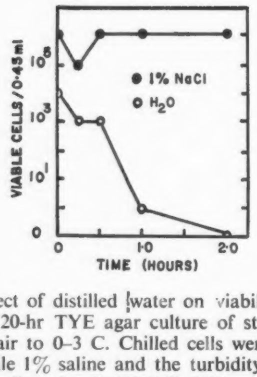

FIG. 3. Effect of distilled water on viability of strain V1 cells. A 20-hr TYE agar culture of strain V1 was adjusted in air to 0–3 C. Chilled cells were suspended in cold, sterile 1% saline and the turbidity of the suspension was adjusted to 0.460 O.D. (420 mμ). An aliquot of this suspension (0.1 ml) was added to 9.9 ml of cold, sterile 1% saline and to 9.9 ml of cold, sterile, triple-glass-distilled water, respectively; at the times indicated the dilute suspensions were thoroughly mixed and assayed for viable cells (see Methods). During the experiment, cell suspensions were maintained at 0–3 C.

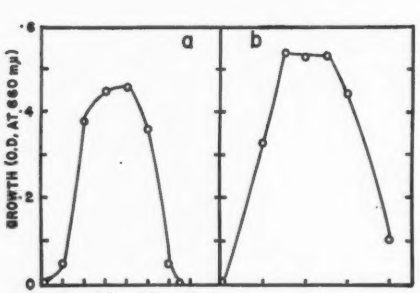

FIG. 4. Effects of temperature and pH on growth of strain V1. Tubes of uniformly inoculated TYE broth (5 ml/tube) were incubated static in water baths at (a) the indicated temperatures or (b) at 25 C at the pH values indicated (pH values were adjusted with 2 N HCl or 2 N KOH before autoclaving and no appreciable pH changes occurred during sterilization). At 20 hr, growth was stopped by adding 5 ml of 2% formaldehyde–1% saline solution to each tube. Values plotted are averages of three replicate determinations and represent turbidity before dilution.

was present as the sole source of carbon. Since, however, they also failed to grow when glucose was the sole carbon source, lack of growth was due to an inability of the organisms to utilize NH_4^+ ion as a source of nitrogen. When the basal medium contained vitamin-free casitone as a source of organic nitrogen, moderate growth resulted. This growth was increased twofold if glucose or citrate was also present, but citrate was only utilized for growth after an extended lag period (10–14 days). Malonate and tartrate were not utilized.

The strains showed moderate catalase activity and gave strongly positive oxidase reactions.

Drug Sensitivity

The organisms were sensitive to the lowest levels tested (5 μg) of the following antibiotics: novobiocin, chloromycetin, aureomycin, and terramycin. They were also sensitive to the vibriostatic agent 0/129 (unlike *A. liquefaciens* and *A. salmonicida*) but were completely resistant to penicillin at the highest concentration tested (10 units).

Pathogenicity

Pathogenicity of the four strains was confirmed using salmon held in fresh water at 14–15 C. When sockeye salmon (four 9-g fish for each strain) were each injected intraperitoneally with 0.1 ml of a 1% saline–0.1% peptone suspension containing approximately 10^7 viable cells (saline–peptone-washed 48-hr cells from TYE agar), all fish were killed within 48 hr. Identical results were obtained with strain V1 and chum salmon (10 fish averaging 7 g/fish). In every instance, the injected strain was reisolated in pure culture from the kidneys of the dead fish, and except for a general absence of diffuse haemorrhages on the body surface, symptoms typical of the natural disease were reproduced. Control fish injected with a variety of materials at levels equivalent to, or exceeding, the dose of viable cells, all survived: saline–peptone; saline–peptone plus cells killed by heating for 5 min at 52 C; cell-free extracts.

Discussion

Vibriosis has been reported from Japan and the United States in fish cultured entirely in fresh water (Hoshina 1957; Kishi et al. 1958; Murae et al. 1959; Hayashi et al. 1964; Saito et al. 1964; Muroga and Egusa 1970; Rucker et al. 1953; Ross et al. 1968), but the disease is primarily a marine phenomenon and the freshwater infections were probably all due to introduced marine vibrios (halophilism of the Japanese strains was overlooked for many years: of the strains tested for this feature, those of Hoshina (see Egusa 1969), Hayashi et al. (1964), and Muroga and Egusa (1970) were clearly halophilic; those of Saito et al. (1964) were reported as nonhalophilic). Like the present strains, *Vibrio* fish pathogens survive poorly in fresh water (Bergman 1909; Schäperclaus 1928; Wolter 1960; Ross et al. 1968), and apparently even the supposedly nonhalophilic strains of Saito et al. (1964) were killed rapidly in this medium (in infected rearing ponds they could only be isolated in the deeper waters where fish dead of the disease had presumably collected). The freshwater form of the disease therefore only appears likely where, as in the feeding of an infected diet, the causative bacterium has little or no contact with the water during transmission, and the real impact of the disease will be felt in marine fish culture, which is now attracting much interest. Here, the water itself will be a continuing potential source of infection, and judging from the experience of others and the present results, unless marine facilities can be operated to maintain water temperatures and dissolved organic matter at reasonably low levels, the disease could prove devastating.

Taxonomically, the present strains were vibrios because in addition to being gram-negative, polar-flagellate, asporogenous, oxidase-positive, anaerogenic, fermentative rods, they produced acetoin, were sensitive to compound 0/129, fermented inositol, liquefied gelatin, and failed to decarboxylate lysine. The last five features listed are not intended as part of a definition of the genus *Vibrio*. They serve only to distinguish the present strains from the otherwise *Vibrio*-like anaerogenic aeromonads and plesiomonads, which not only fail to produce acetoin but are either resistant to compound 0/129 and fail to ferment inositol (*A. formicans*) or fail to liquefy gelatin but decarboxylate lysine (*Plesiomonas shigelloides*) (Bain and Shewan 1968; Carpenter et al. 1968).

The strains were identified as *V. anguillarum* as they closely resembled the strain of this species isolated by Bagge and Bagge (1956). Fuller descriptions are available for this strain than exist for the original strain (inconsistently described by Bergman in 1909 and 1912) or for strains studied by other early (Schäperclaus 1928, 1934; Nybelin 1935) and recent (see Table 2) authors, and its adoption as a reference culture was considered justified since it appeared representative of these *Vibrio* fish pathogens in its biochemical (Table 2) and other features (Kiehn and Pacha (1969) regarded it as "archtypical" of the strains they studied from the Pacific Northwest and Europe). Of 70 or so morphological, cultural, and biochemical

ABLE 2. Some frequently reported biochemical properties[a,b] of *Vibrio anguillarum*-like bacteria isolated from diseased fish and shellfish (1950–70). Symbols: +, positive reaction; - action; ·, information not given in source cited; D, different strains gave different reactions in hands of one worker; fractional figure after D, number of strains positive/number te onal figure after + or −, number of authors reporting + or −/number of authors reporting the test; V, 20% or more of the strains gave reactions different from that indicated for the chtype" of *V. anguillarum*.

train(s) and designation if represented in the National Collection of Marine Bacteria (NCMB), Torry Research Station, Aberdeen, Scotland	Bagge & Bagge 1956 NCMB 6	Present strains	Hodgkiss & Shewan 1950 NCMB 407	Hoshina 1956, 1957 NCMB 571, 572, & others	Kishi et al. 1958	Murae et al. 1959	NCMB 828	Wolter 1960	Smith 1961 NCMB 829	NCMB 1291	Hayashi et al. 1964	Saito et al. 1964	Kusuda 1965[a]	Lagarde & Chakroun 1965	Muroga & Egusa 1967	Ross et al. 1968	Cisar & Fryer 1969	Pacha & Kiehn 1969	Muroga & Egusa 1970	Tubiash et al. 1970 Type II, 4 strains	Emerging archtype[c] of *V. anguillarum*	Remark
ermentative (glucose)	+	+	+	+	·	+	+	·	+	+	·	·	·	+	+	+	+	+	+	+	+	
ias from glucose	−	−	−	−	−	−	−	−	−	−	−	−	·	−	−	−	−	−	−	−	−	
xidase (Kovacs)	+	+	+	+	·	·	+	·	+	+	·	·	·[d]	·	·[d]	+	·[d]	·	·[d]	+	+	Presumptive tes brio genus (She
ensitive to: 0/129	+	+	+	+	·	·	+	·	+	+	·	·	·	·	+	+	+	·	+	+	+	
penicillin	−	−	−	−	·	·	·	·	−	−	·	−	·	·	−	·	·	−	·	+	−	
cid from:																						
Fructose	+	+	+	+	+	+	·	·	+	·	+	+	+	+	+	+	+	+	D6/7	·	+	
Galactose	+	+	+	+	+	+	·	·	+	·	+	+	+	+	+	+	+	+	−	·	+	
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Mannose	+	+	+	+	+	+	+	·	+	+	+	+	+	+	+	+	+	+	D2/3	·	+	
Maltose	+	+	+	+	+	+	+	+	+	·	+	·	+	+	−[i]	+	+	+	+	+	+	
Trehalose	+	+	+	+	+	·	+	·	+	+	+	·	·	·	·	+	+	·	·	·	+	
Dextrin	+	+	+	+	+	·	·	·	+	·	+	·	+	·	+	·	+	·	+	·	+	
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	·	+	+	·	+	+	·	+	
Sucrose	+	+	+	+3/4	+	+	+	+	+	+	+	+	−	−	+	+	+	D11/12	+	+	+	
Glycogen	+	+	+	+	·	·	·	·	+	·	+	·	+	·	D3/6	·	+	·	·	·	+	
Starch	−[e]	+	−[e]	+[e]	·	·	·[e]	·	+[e]	·[e]	+	·	+	+	+[e]	·[e]	·[e]	·[e]	+[l]	·[e]	+V	
Sorbitol	+	+	+1/2	D[f]	+	+	+	·	+	+	−	·	−	−	·	+	·	·	−[l]	·	+V	
Glycerol	+	+	+	−	·	·	·	·	+1/2	·	−	+	·	−	D2/6	+	+	+	−[j]	·	+V	
Cellobiose	+1/2	+	+1/2	+2/3	·	·	−	·	−	−	−	·	+	·	D5/6	·	+	·	D5/6	·	+V	
Arabinose	+3/4	−	−	+	+	−	−	·	−	−	+	+	−	−	D3/6	+	·	D11/12	D5/6	·	+?V	
Lactose	−	−	−	−	+	+	−	+	−	−	+	−	−	−	−	−	−	D 2/12	−[l]	−	−V	
Inulin	−	−	−	−	−	−	·	·	−	·	−	−	·	·	·	·	+	·	·	·	−	
Inositol	−	+	−	−3/4	−	·	−	·	−	−	−	−	−	−	D5/6	·	−	·	−	·	−	
Rhamnose	−	−	−	−	−	·	−	·	−	−	−	·	−	−	D2/6	−	−	·	−[l]	−	−	
Xylose	−	−	−	−	−	+	−	·	−	−	−	−	−	·	−	−	−	−	−[k]	·	−	
Raffinose	−	−	−	−	−	·	−	·	−	−	−	−	−	·	−	−	−	·	−[l]	·	−	
Adonitol	−	−	−	−	−	−	−	·	−	−	−	·	−	−	·	−	·	·	·	·	−	
Dulcitol	−	−	−	−	−	−	−	·	−	−	−	−	−	−	·	−	−	·	·	·	−	
Salicin	−	−	−	−	−	·	−	·	−	−	−	·	·	·	·	−	−	·	·	·	−	
atalase produced	+	+	+	+	·	·	+	+	+	+	+	·	+	·	+	+	+	+	+	+	+	
itmus milk peptonized	+	+	+	+	·	·	·	·	+	·	+	+	·	·	·	·	+	·	·	·	+	
litrate reduced	+	+	+	+	+	+	+	·	+	+	+	+	+	+	+	+	+	+	+	·	+	
ielatin hydrolyzed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D10/12	+[j]	+	+	
rginine attacked	+	+	+	+	·	·[h]	+	·	+	+	·	·	·	−	+	·	·	·	D5/6	+	+	
ndole produced	+4/5	+	+	+[f]2/3	+	+	+	+	+2/3	−	+	+	+	−	+	−	+	D11/12	+	D[m]?	+V	
cetoin produced	+	+	−[g]	+3/4	+	−	+	·	+	+	−	−	−	−	+	+	+	D 9/12	+	−	+V	
itrate as sole C-source (growth)	+3/4	−	−	−2/3	+	−	−	·	−2/3	−	−	·	+	+	+	D	−	D11/12	+	−	−V	Ability to attac may be of significance fo pathogenic fo therms (see Ba wan 1968)
lethyl red reaction	+1/2	−	+1/2	−2/3	+	·	·	·	−	·	−	−	+	−	−	+	−	·	D4/6	·	−V	
iluconate oxidized	+1/2	+	−	−	·	·	−	·	−	−	·	·	·	·	·	−	·	·	·	−	−V	
lalonate utilized	−	−	−	−	·	·	−	·	−	−	−	·	·	·	+	−	·	·	+	·	−	
ellulose attacked	−	−	−	−	·	·	·	·	−	·	·	·	·	−	·	·	−	·	·	·	−	
lydrogen sulphide produced	−	−	−	−[f]	−	·	−	·	−	−	−	−	−	−	−	−	−	−	−	·	−	
ysine decarboxylated	−	−	−	−	·	·[h]	−	·	−	−	·	·	·	−	−	·	·	·	D1/6	−[n]	−	
rnithine decarboxylated	−	−	−	−	·	·	−	·	−	−	·	·	·	−	·	·	·	·	·	−	−	
henylalanine deaminated	−	−	−	−	·	·	−	·	−	−	−	·	·	−	·	−	·	·	·	·	−	
Jrease produced	−	−	−	−	−	·	−	·	−	−	−	−	−	·	−	·	−	·	·	·	−	

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[a] Data for strain NCMB 6 from (1) Thornley 1960, (2) Smith 1961, (3) Shewan 1963, (4) Ross et al. 1968, (5) Sakazaki 1967 as cited by Egusa 1969, (6) Pacha and Kiehn 1969, nd Bagge 1956. Data for NCMB 407 from (1), (2), (3), (5), and Hodgkiss and Shewan 1950. Data for Hoshina's strains from (2), (3), (5), Kishi et al. 1958, and Hoshina 1956, 195 NCMB 828 (from dead *Salmo salar*, River Foyle, Ireland) and NCMB 1291 (from moribund saithe, Torry Research Station, Scotland) from (5). Data for NCMB from (2), (3), (5 Data for Muroga and Egusa's strains from Muroga and Egusa 1967, 1970, and Egusa 1969. Data for Kusuda's strains from Egusa 1969. Data for other strains from original autho

[b] Most authors give little information about the tests used, and because the strains grew with the salt concentrations present in most conventional media, the salt requirement was ometimes overlooked, or a salt supplement was thought unnecessary. Some authors used added salt, however, in some (Hayashi et al. 1964; Cisar and Fryer 1969) or all (Muroga 967; Ross et al. 1968; Pacha and Kiehn 1969) of their tests, and the fact that the outcome of some tests could be influenced by salt concentration (clearly demonstrated by pres nd those of Ross et al. 1968) probably accounts, in part, for some of the variability and inconsistencies reported.

[c] Archtype is used here to denote a hypothetical organism possessing features given by a majority of its constituent strains.

[d] Kovacs' oxidase test not reported but strains were oxidase-positive by another method.

[e] Starch was hydrolyzed.

[f] Initially, Hoshina (1956) reported that his strains produced H_2S and indole and fermented sorbitol. Without special comment, he later stated that sorbitol was not fermented and ome of his strains produced H_2S and indole (trace amounts). Other authors who studied strains obtained from Hoshina confirmed that H_2S was not produced (Kishi et al. 1958; Sakazaki 1967 as cited by Egusa 1969), and showed that sorbitol was fermented (Kishi et al. 1958; Smith 1961), although only by some strains (Sakazaki 1967 as cited by Egusa 196 was again inconsistently reported (Kishi et al. 1958; Sakazaki 1967 as cited by Egusa 1969 reported a positive test; Smith 1961 reported a negative test).

[g] Acetoin is apparently produced, but it is all reduced to 2,3-butanediol (Sakazaki 1967 as cited by Egusa 1969).

[h] Nongrowing ("resting") cells in a Warburg respirometer exhibited strong arginine, and weaker lysine, decarboxylase activity.

[i] Maltose fermentation has been inconsistently reported for these strains (compare Muroga and Egusa 1967, 1970, with Egusa 1969).

[j,k,l] Only 3, 4, and 6 (j, k, and l, respectively) of the seven strains described by Muroga and Egusa (1970) were tested for these reactions.

[m] It is uncertain whether the four strains listed here differed in indole production. Tubiash et al. (1970) did not give information on individual strains; their information was given f of seven pathogenic vibrios, four of which they identified as *V. anguillarum*.

[n] Twedt et al. (1969) studied one of these strains and reported it as lysine decarboxylase-positive.

characters presently available for comparison, the strain of Bagge and Bagge differed from the present strains in only five or six: it grew slowly at 37 C; it grew with citrate as a sole source of carbon; it produced acid from arabinose but not from starch or inositol, and it gave a possible positive methyl red reaction.

On the basis of the indole reaction and the production of acid from sucrose and mannitol, the species *V. anguillarum* has to date been divided into three biochemical types (A, B, and C) (Nybelin 1935; Smith 1961). According to this system of typing, the present strains belong to *V. anguillarum*, type A (they were positive in all three tests listed). This system of typing, however, might now be discontinued. In early studies, the system was of value because a classification as to type told a fair proportion of what was known about the organism. Also, because the system stressed the existence of variability within the species, it had the added advantage of forestalling the creation of new species based on a few biochemical differences. Today, however, bacterial classification is routinely based on a much larger number of features than was formerly used and the advantages of retaining the typing system appear less obvious. Additionally, it is extremely doubtful whether any significance can be attached to a system of typing based on indole production. This feature has now been inconsistently reported for several strains (Table 2) indicating either that it is an unstable feature, or that a reliable indole test has yet to be devised for these organisms.

If *V. anguillarum* is ever to be formally recognized as a species in Bergey's Manual it would appear expedient to view the strains in Table 2 as variants of this species. Despite the variability, inconsistencies, and gaps in the descriptions available, the strains are very similar to each other and to the strains of this species studied by Bergman (1909, 1912), Schäperclaus (1928, 1934), and Nybelin (1935), and in fact, comparative studies with some of the strains listed in Table 2 have consistently supported the idea that they constitute a single species (Smith 1961; Sakazaki 1967 as cited by Egusa 1969) or recognizable group (Bain and Shewan 1968). Species status might also be more readily achieved if pathogenicity as a criterion of the species were demoted in importance. Pathogenicity is a notoriously unstable feature and one that requires special facilities to demonstrate; moreover, since strains of *V. anguillarum* are probably saprophytes that are facultatively pathogenic, they are likely to be found in a variety of marine situations, including healthy marine animals (Tubiash et al. 1965, 1970).

On the basis of features that are emerging as more or less stable, it is now possible to construct a tentative archtype for *V. anguillarum* that would distinguish it from all, or most, of the proposed and presently recognized vibrios. The former category would include such marine organisms as the cephalotrichous, seawater-requiring, fish pathogen *Pseudomonas ichthyodermis* (originally *Achromobacter ichthyodermis*, Wells and ZoBell 1934), which is now classified as a *Vibrio* (Shewan 1963), and *V. parahaemolyticus*, an organism primarily of public health interest, but one that can apparently also cause disease in marine animals (Krantz et al. 1969); the latter category would contain the vibrios listed in Bergey's *Manual of Determinative Bacteriology* (Breed et al. 1957) (based on carbohydrate metabolism, some of these are now regarded as pseudomonads (Shewan 1963), and another, *V. piscium*, is of uncertain generic status).

A suggested archtype for *V. anguillarum*, in addition to having the biochemical properties listed in Table 2 (right-hand column), would also be a facultatively anaerobic, nonsporing, actively motile, polar monotrichous flagellate, gram-negative rod; capable of growth on conventional media with salt levels as low as about 0.07% NaCl but growing optimally with 1–3% NaCl; growing well, or best, at 20–30 C, and poorly, or not at all, at 37 C; nonluminescent; unable to digest agar; may sometimes cause fish disease.

It remains to be seen, however, whether the features suggested for *V. anguillarum* will prove stable and distinctive enough to prevent its possible confusion with other marine vibrios. Many vibrios in the latter group are commensal with marine animals (Leifson et al. 1964; Lovelace et al. 1968) and detailed information on these organisms is only now becoming available (Sakazaki 1967 as cited by Egusa 1969; Twedt et al. 1969). Ideally, therefore, before *V. anguillarum* is formally recognized as a species, a comparative study employing as many as possible of the available strains from both groups is indicated. Hopefully, a growing interest in marine fish culture will provide the necessary impetus for such a study.

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Upper Lethal Temperatures in Relation to Osmotic Stress in the Ribbed Mussel *Modiolus demissus*[1]

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WAUGH, D. L., AND E. T. GARSIDE. 1971. Upper lethal temperatures in relation to osmotic stress in the ribbed mussel *Modiolus demissus*. J. Fish. Res. Bd. Canada 28: 527–532.

Upper lethal temperatures for the ribbed mussel *Modiolus demissus* (Dillwyn) were determined for thermal acclimations of 15.0, 20.0, and 25.0 C and osmotic acclimations at 5, 15, and 28‰ S, which yielded nine combinations. These upper lethal temperatures for 1440 min exposure ranged from 38.42 to 40.18 C, generally paralleling increases in thermal acclimation. The upper lethal temperature within any thermal acclimation decreased as the salinity of the bioassay departed from the appropriate level of osmotic acclimation. Depression of the upper lethal temperature was more pronounced in the test medium of 5‰ S. The shift from osmoconformity to osmoregulation, which occurs in this species when the ambient salinity is reduced to 350 mosmols (approximately 10‰ S) apparently extends the metabolic load or stress relative to that induced by similar temperatures in higher levels of salinity. Order of death in any bioassay was independent of size expressed as length, and specific sex.

The upper lethal temperatures are higher than the mean temperature of the substrate of the collecting site during the warmest period of the summer, but are approximately the same as the maximum substrate temperature during that period. Survival of ribbed mussels in such potentially lethal conditions is explained by the shorter intermittent natural exposures than those employed in the bioassays.

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THE ribbed mussel *Modiolus demissus* (Dillwyn) is a pelecypod ranging widely from middle temperate latitudes to the tropics. Because of its local distributions high in the intertidal zone, the ribbed mussel is often exposed to air for several consecutive days at times of asymmetrical tides that result in prolonged low levels of water, thereby exposing it to some extended periods of intense insolation. Salinity in estuaries and salt marshes also may fluctuate considerably. Because of such relative instability of prime environmental variables, ribbed mussels can be expected to be frequently in states of *stress*, which have been defined by Brett (1958). Degree of stress is manifest in the degree of physiologic tolerance to excessive levels of these variables. The measure of tolerance as defined by Fry (1947) is the *incipient lethal level.*

In this study, lethal temperatures in the ribbed mussel are measured in relation to various states of osmotic performance and in relation to various states of thermal and osmotic acclimation. The results are related to thermal conditions that exist in habitats near the northern limit of their zoogeographic range.

Methods and Materials

Ribbed mussels were collected from an intertidal bed, located on Bideford River, near the Fisheries Research Board of Canada, Oyster Unit, at Ellerslie, P.E.I. This population was located in a dense growth of the intertidal grass *Spartina alterniflora*. The mussels at the lower edge of the bed were exposed to air, on the average, 42% of the time whereas those at the upper edge were exposed to air for 78% of the time, which was considerably greater than that for any other local pelecypod (Thomas MS 1970). A thermorecorder was maintained at the collecting site.

ACCLIMATION

The first series of experiments on mussels acclimated at 28‰ S and temperatures of 15.0, 20.0, and 25.0 C was performed at the Ellerslie Laboratory. The mussels were acclimated in polyethylene trays 42 × 70 × 10 cm, each being supplied with running sea water that was maintained at one of each of these temperatures. The remaining experiments in which mussels were acclimated to 15 and 5‰ at thermal acclimations of 15.0, 20.0, and 25.0 C respectively were performed in a laboratory of the Department of Biology, Dalhousie University. The acclimation apparatus consisted of three 75-liter aquaria. Each bath was controlled by a

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[1] Based on a thesis submitted by D.L.W. in partial fulfillment of the requirements for the degree of Master of Science from Dalhousie University.

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thermoregulator sensitive to changes of 1 degree C. In all experiments, mussels were held at the appropriate temperature and salinity for at least 8 weeks during which a progression of bioassays was conducted on each series of samples.

BIOASSAYS

Bioassays were performed in glass aquaria, 44 × 22 × 28 cm, containing 22 liters of water. Temperatures were maintained with heaterthermostats, accurate to –0.1 degree C. All tests in each bioassay were conducted for 1440 min. Ten measured animals were placed in a test bath that had been previously adjusted to one of the selected series of experimental temperatures. Tests were repeated, using various constant temperatures at intervals of 0.2 degree C, until a range of mortalities from 10 to 90% could be determined. Upon termination of each test, the animals were removed from the bath, placed in small perforated polyethylene containers, and were then returned to the appropriate acclimation bath for 24 hr. This procedure permitted the animals to recover if possible. The number of dead mussels was then determined. A mussel was considered dead if the mantle failed to contract when prodded with a glass rod. The sex of each mussel was then determined by examination of the gonads.

Since mussels were acclimated at all designated combinations of temperature (15.0, 20.0, and 25.0 C) and salinity (5, 15, and 28‰), there were nine *series* or populations, and since bioassays were conducted at three salinities, there were 27 *subseries* (Table 1). Initially, upper lethal temperatures were determined on nine of these subseries, in media corresponding to the three levels of osmotic acclimation for each of the three levels of thermal acclimation. Subsequently, tests were performed on the remaining 18 subseries in media corresponding to the other levels of osmotic acclimation. Bioassays of subseries were conducted fortnightly, beginning 14 days after the commencement of acclimation, and were continued until the lethal temperatures of two consecutive bioassays in any subseries were calculated to be within 0.05 degree C.

OSMOMETRY

Five groups of 30 mussels each were placed in dilutions of sea water of approximately 5, 8, 10, 15, and 28‰ S at 15 C. After acclimation for 10 days, the osmolality of the haemolymph of four mussels from each group and of the respective media were determined using an "Osmette" direct-reading osmometer.

STATISTICAL PROCEDURES

The statistical procedure used to determine the upper lethal temperature in each bioassay was probit analysis, in which temperature is expressed arithmetically and percent mortality is expressed as a probit (Bliss 1938). A regression analysis was then performed on data for a particular bioassay. The temperature that produced 50% mortality, as determined from the regression equation, was recorded as the upper lethal temperature.

Probability analysis, adopted from Harding (1949), was used to describe the length distributions of these mussels in relation to order of death and to differential mortality within sexes.

Results

The upper lethal temperature for each subseries is shown in Table 1. The highest upper lethal temperature, 40.18 C, occurs in those mussels having

TABLE 1. Upper lethal temperatures for 24-hr exposures of samples of ribbed mussels (*Modiolus demissus*) acclimated at temperatures of 15.0, 20.0, and 25.0 C in ambient salinities of 5, 15, and 28‰ (series) and subsequently subjected to thermal bioassay at each of these levels of salinity (subseries). The number of tests performed at any salinity corresponding to the level of osmotic acclimation is always greater than those in the remaining portion of the respective series since each was the initially conducted subseries employed to determine an approximation of the time interval for complete thermal acclimation. After the completion of thermal acclimation had been established, relatively few tests were required to fix the lethal temperatures in the remaining respective subseries. Upper lethal temperatures are given with 99% fiducial limits.

Acclimation salinity (‰)	Acclimation temp (C)	Bioassay salinity (‰)	No. tests	Upper lethal temp (C)
28	15.0	28	28	39.63±0.09
		15	8	38.82±0.07
		5	7	38.79±0.05
	20.0	28	31	39.51±0.07
		15	7	38.79±0.05
		5	8	38.59±0.04
	25.0	28	31	40.18±0.06
		15	5	38.98±0.09
		5	4	39.10±0.01
15	15.0	28	9	38.22±0.06
		15	49	39.09±0.05
		5	7	38.40±0.02
	20.0	28	8	38.50±0.10
		15	30	39.05±0.06
		5	7	38.19±0.05
	25.0	28	8	39.35±0.17
		15	29	39.39±0.12
		5	7	37.43±0.09
5	15.0	28	6	38.20±0.06
		15	8	38.54±0.09
		5	35	38.42±0.04
	20.0	28	8	37.79±0.04
		15	5	38.37±0.10
		5	30	38.98±0.09
	25.0	28	6	37.97±0.12
		15	8	38.41±0.04
		5	32	38.73±0.05

an acclimation of 25.0 C and 28‰ S. Upper lethal temperatures decrease with decreasing levels of thermal and osmotic acclimation (Fig. 1). The lowest upper lethal temperature, 38.42 C, corresponds to an acclimation of 15.0 C and 5‰ S.

Susceptibility to death by exposure to higher temperatures was not dependent upon size since there is no appreciable departure between the curves of size distributions for the dead and for the population in any of the nine series. An example typical

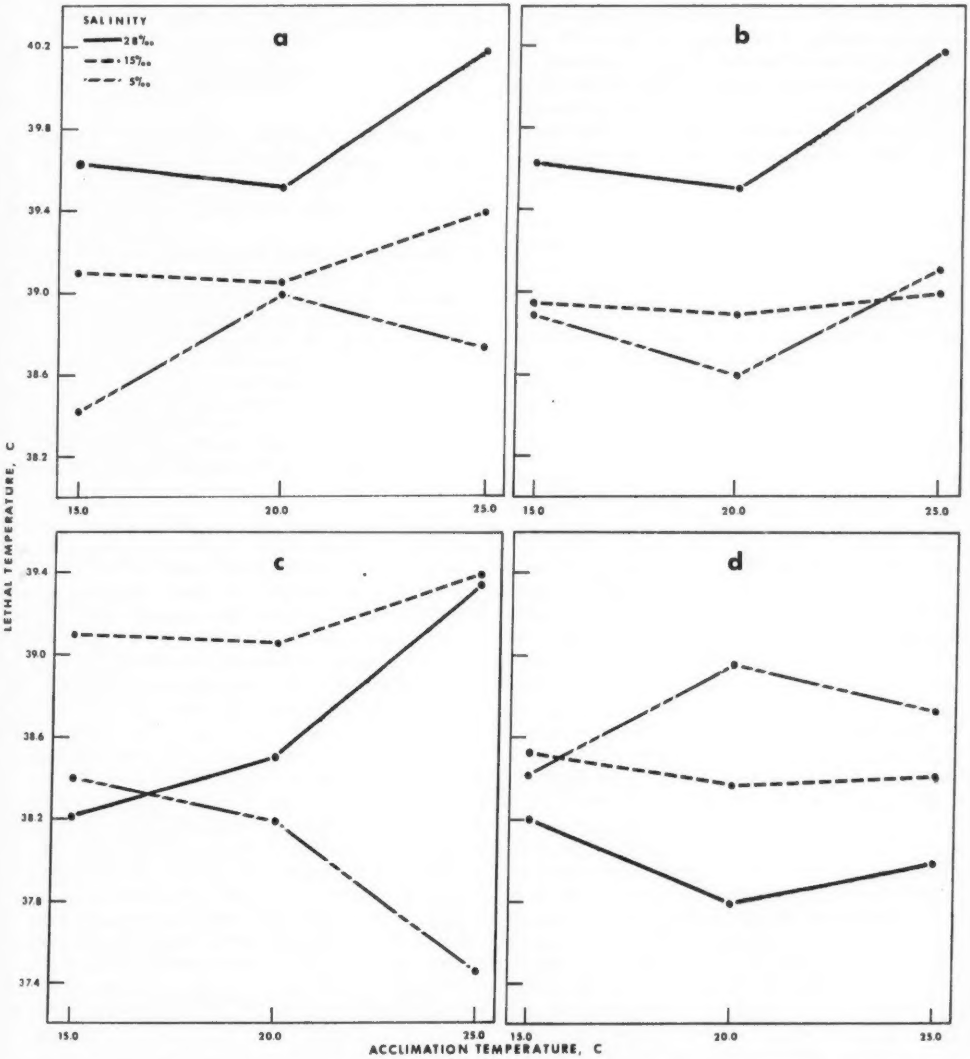

FIG. 1. Upper lethal temperature relations of ribbed mussels tested at salinity levels of 5, 15, and 28‰, for thermal acclimations of 15.0, 20.0, and 25.0 C. Figure 1*a* describes these relations in samples tested at salinities corresponding to the respective levels of osmotic acclimation (5, 15, 28‰). Lethal temperatures for osmotic acclimations of 28, 15, and 5‰ S are shown in (*b*), (*c*), and (*d*) respectively.

of this independence is shown in Fig. 2 for ribbed mussels acclimated at 15.0 C and 5‰ S. The remaining eight series also lack any relation between size and order of death (Waugh MS 1970).

The relation of the size distributions of each sex in a series of samples is shown in Fig. 3, and the separate size distributions of females and males that died following experimental exposure are presented in Fig. 4.

The curve for osmometry (Fig. 5) indicates that sea water and haemolymph of the ribbed mussel at 15 C are isosmotic from 750 to 350 mosmols/kg but that the haemolymph becomes hyperosmotic to external concentrations below 350 mosmols/kg.

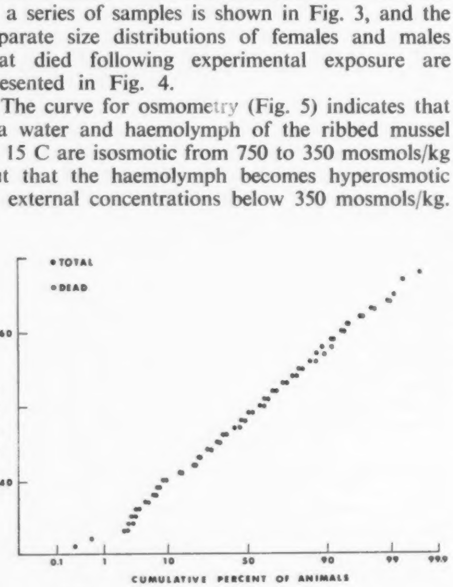

FIG. 2. An example of the relation between size distribution in the entire experimental population of ribbed mussels (solid circles) and the size distribution of those that were acclimated at 15.0 C and 5‰ S and that died during experimental exposures (open circles).

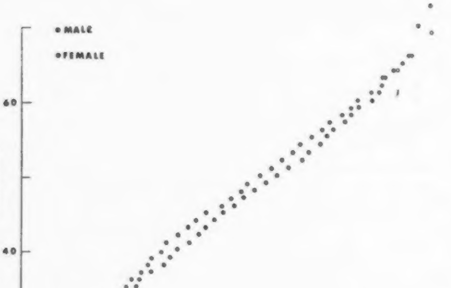

FIG. 3. An example of size distribution of male and female ribbed mussels. This distribution represents all ribbed mussels that were acclimated at 28‰ S and 15.0, 20.0, and 25.0 C, before experimental exposures.

Recordings of the substrate temperature at the collection site indicate that the surface temperature of the exposed mussel bed may exceed 40 C at times when air temperature ranged from 25 to 30 C. Such temperatures not uncommonly persisted for several hours daily during July and August. Salinity in the area is usually about 28‰, corresponding to the highest level used in these bioassays.

Discussion

In general, in aquatic poikilotherms, a decrease in environmental salinity is accompanied by a decrease in heat tolerance (Kinne 1956, 1958). The present results confirm this trend in the ribbed mussel.

The highest upper lethal temperature recorded for ribbed mussels in this study, 40.19 C, is probably somewhat lower than their *ultimate* upper lethal temperature since it is obvious that they could

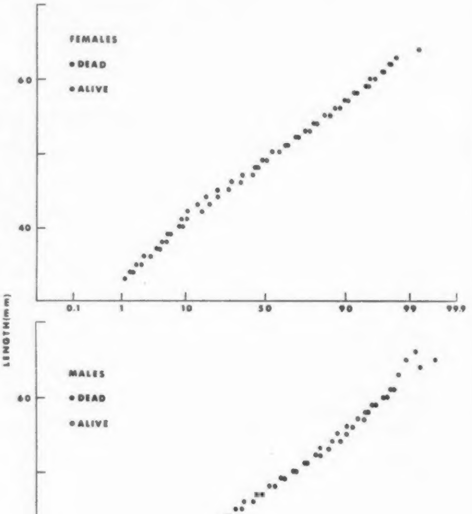

FIG. 4. An example of the relation between the size distributions of dead and surviving ribbed mussels segregated according to specific sex in the population acclimated at 28‰ S and 15.0, 20.0, and 25.0 C, prior to experimental exposures (see Fig. 3).

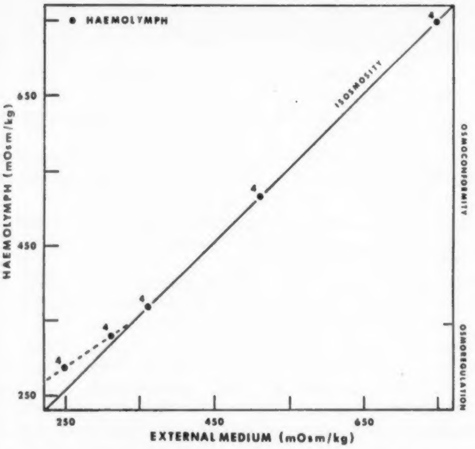

FIG. 5. Mean osmotic concentrations of haemolymph (mosmols/kg) of ribbed mussels expressed as functions of the osmosity of various dilutions of ambient sea water at 15 C. The inflection of the lower segment of the curve marks the level of the medium, 350 mosmols/kg or 10‰ S, at which ribbed mussels shift from osmoconformity to osmoregulation. The numeral above each point indicates the number of determinations.

have been acclimated in water of 28‰ S to temperatures above those lethal values for water having lower salinities. Thus, for example, data in Table 1 indicate that thermal acclimation should be possible to at least 37 C, and this could be expected to enhance tolerance even further. Read and Cumming (1967) report that the upper limit of thermal tolerance of this species, when the temperature is raised by increments of 1 degree C each 3.5 days in sea water (35‰ S) is 40 C. Vernberg et al. (1963) reported that the lethal temperature of gill tissue from ribbed mussel acclimated at 25 C and 30‰ S was 43.5 C using 150-min exposures in medium of 30‰ S. Lent (1968) states that the LD50 for ribbed mussel ranges from 36.4 to 37.8 C when they are subjected to a 10-hr exposure in air at 80% relative humidity. Although different techniques were used to obtain these data on upper lethal temperatures for ribbed mussel, they are somewhat similar to the present results. However, further comparison cannot be made since details of other acclimations are incompletely reported.

Ribbed mussels, unlike most pelecypods whose osmotic performance has been recorded at various salinities (Robertson 1949, 1953), are osmoconformers at higher levels but become osmoregulators below 10‰ S. Osmoregulation in common with other activities can elicit a supranormal metabolic response and so initiate a state of stress. Such stress probably accounts for the shift in the expected pattern of lethal response at the lowest test salinity (5‰) in which the lethal temperature was lower with the highest thermal acclimation than with the intermediate level (Fig. 1*a*). Depressions of lethal temperature that occurred in test solutions other than those of the respective osmotic acclimations are tentatively explained as being the result of shifts to reduced metabolic efficiency during adjustments to new osmotic conditions, whether these adjustments are those of relatively passive conformance or of active regulation, the latter activity being rather more stressful.

Relations between body size and thermal tolerance are not as thoroughly documented for invertebrates as they are in the poikilothermic vertebrates in which there is generally no influence of size (Hart 1952; Brett 1970). McLeese (1956) reported in his extensive study that size does not alter the tolerance of lobster (*Homarus americanus*). Results of the present study indicate that there is no correlation between thermal tolerance and size, at least within the size range of animals used. Figure 4 indicates that no size-dependent mortality resulted from thermal stress in either males or females of the population considered.

Tissue temperatures of exposed intertidal animals are frequently higher than those that would be expected from local intensities of insolation (Gunn 1942; Southward 1958; Lewis 1963). Since surface temperatures of the ribbed mussel bed frequently exceeded 40 C, tissue temperatures could be expected to approach such a value or perhaps exceed it. However, a separate exposure to such high temperatures is for a rather shorter interval than that used in these bioassays. Although these temperatures at the peak of summer warming probably exceed the upper incipient lethal temperature for the acclimatization in this natural population, survival of these mussels indicates that such potentially lethal exposures must be of shorter duration than the period of thermal resistance. Unfortunately, resistance times could not be determined in the present study. The method of calculating upper lethal temperatures for mussels employs tests of fixed duration in which the extent of mortality can be determined only at the end of the test. Since it is not possible to observe the moment of death of a mussel, as it is for example in fish, median resistance times cannot be computed by the method of Fry et al. (1946) and therefore, bioassays would be required from a broad array of fixed exposure times.

Acknowledgments

We express our appreciation to Dr M. L. H. Thomas, who provided valuable advice during the early phases of the research, and to Mr R. E. Drinnan and staff at the Fisheries Research Board of Canada, Oyster Unit, Ellerslie, P.E.I., who provided space and equipment as required. The study was assisted by a Fisheries Research Board of Canada graduate scholarship to D. L. Waugh. Equipment was provided from a National Research Council operating grant to E. T. Garside.

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Chondrococcus columnaris Disease of Fishes: Influence of Columbia River Fish Ladders

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FUJIHARA, M. P., AND F. P. HUNGATE. 1971. *Chondrococcus columnaris* disease of fishes: influence of Columbia River fish ladders. J. Fish. Res. Bd. Canada 28: 533–536.

Columbia River fish ladders were surveyed as a possible ecological niche for *Chondrococcus columnaris*, the causative agent for columnaris disease of fishes. Water samples from Bonneville, McNary, Priest Rapids, and Rocky Reach fish ladders during periods of warm-water temperature and high population density of fishes showed *C. columnaris* levels of from 0.06 to 4.1 organisms/ml. Samples taken upstream or downstream from the ladders were consistently negative. Average agglutinating titers and incidence of *C. columnaris* infection in largescale suckers were higher at the Bonneville ladder than at a site several miles downstream. Coho salmon from an upper-river fish ladder (Rocky Reach) showed a higher incidence of infection and greater antibody production than at the downriver Bonneville ladder. It is concluded that fish-ladder facilities on the Columbia River are important sites for repetitive exposure of anadromous salmonids to *C. columnaris*.

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MORTALITIES in salmonid populations induced by *Chondrococcus columnaris* are usually associated with warm river water temperatures (Fish 1948; Pascha MS 1961; Colgrove and Wood 1966). Increasing use of the Columbia River for agriculture, industry, power, etc., can change the temperature characteristics of the river in a manner favorable to increased growth of *C. columnaris* and proliferation of the disease. It is necessary, therefore, that we examine the existing environmental factors that contribute to the disease in the river.

Proliferation and release of *C. columnaris* from fish ladders was demonstrated by the large number of organisms isolated from the Rocky Reach fish ladder on the Columbia River during 1965 (Pauley et al. 1966). The authors found that *C. columnaris* release into the water was related to the high incidence of infected coarse fishes and anadromous salmonids in the ladder. In this report, we describe results that suggest that fish ladders may be important sites for transferring the disease from fish indigenous to the Columbia River to migrating anadromous salmonids in the ladder.

Materials and Methods

Monthly samples of surface water were obtained in the forebay behind the dams, in the top (inlet) and bottom (outlet) of fish ladders, and downstream from the dams. Fifteen separate 1-gal samples were drawn from each ladder and river location and four 0.25-ml aliquots from each gallon were surface plated on solid growth medium. The growth medium was composed of 0.4% Bacto-tryptone, 0.04% Bacto-yeast extract, and 1% Noble agar at *p*H 7.3. Plates were incubated at 22–25 C for 48 hr before counting.

Scraping from gills or necrotic areas, or both, if present, and scrapings from the kidney were related to determine degree of exposure or infection in fish populations. To differentiate between exposure or infection of fishes, the following arbitrary definitions were used: 1–10 *C. columnaris* external isolates were designated as an exposure, and more than 10 external isolates or one or more internal isolates as an infection. A standard agglutination test of fish sera was used as an additional technique to identify prior or recent infection and the magnitude of antibody production. Blood sera for agglutination studies were taken using dorsal aorta punctures (Schiffman 1959).

Results

The seasonal variation in the proliferation and release of *C. columnaris* from fish ladders is illustrated in Table 1. Monthly water samples from the forebay, several hundred feet upstream from the fish ladders, were consistently negative. Since no organisms could be isolated from the forebay water, the organisms found near the ladder inlets were probably released from fish in the uppermost part of the ladder. The values obtained at the ladder outlets represent a total release of organisms from both transient and resident fishes. Water samples

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TABLE 1. Distribution of *Chondrococcus columnaris* release in monthly water samples at Bonneville, McNary, Priest Rapids, and Rocky Reach fish ladders, 1966.

Ladder	Sampling date	River temp (*C*)	Forebay (*isolates/ml*)	Ladder inlet (*isolates/ml*)	Ladder outlet (*isolates/ml*)	Downstream from ladder (*isolates/ml*)
Bonneville	1/23–3/16	4.0–6.7	0	0	0	0
	4/26	11.7	0	0	0	0
	5/18	12.2	0	0	0.06	0
	6/16	13.9	0	0	0.40	0
	7/16	17.8	0	0.20	0.53	0
	8/24	18.9	0	0	0.47	0
	9/27	18.9	0	0.20	1.47	0
	10/24	13.3	0	0.70	0.67	0
McNary	4/29	10.5	0	0	0	0
	6/18	14.4	0	0	0.13	0
	7/28	17.8	0	0.40	1.47	0
	8/25	19.4	0	0.06	1.07	0
	9/29	21.1	0	0.47	1.80	0
	10/29	13.3	0	0.06	0.47	0
Priest Rapids	7/13	15.0	0	0.67	4.13	0
	8/4	16.1	0	0.13	1.60	0
	9/17	16.7	0	0	1.80	0
	10/15	16.7	0	0	0.67	0
	11/5	11.1	0	0	0.20	0
Rocky Reach	10/8	15.3	0	0	1.0	0
	11/14	11.7	0	0.13	0.27	0

1 mile downstream were consistently negative, regardless of month, indicating a substantial dilution of organisms in the mainstream.

No organisms were isolated from either end of the Bonneville ladder during the winter months after dewatering of the ladder, when population density of fishes was minimal and water temperatures were below 11.7 C. Increased temperatures and a noticeable increase in population density of fishes, starting in May, as noted by daily fish counts (U.S. Army Engineers 1966), corresponded with increased releases of *C. columnaris*.

Evidence for *C. columnaris* exposure and infection among fishes in ladders was further demonstrated by comparing largescale suckers (*Catostomus macrocheilus*) from the North Shore Bonneville ladder and from a sampling site several miles downstream from the dam. The results of this study are summarized in Table 2. The higher *C. columnaris* antibody titers and larger incidence of exposure and infection in largescale suckers obtained from the ladder suggest that many of these fish reside in the ladder for extended periods. The data also illustrate the relation between antibody titer and the incidence of *C. columnaris* infection.

If the interpretation of these results is correct, a serious *C. columnaris* infection problem could result from the passage of anadromous salmonids through ladder facilities with heavy concentrations of infected fishes. This suggestion is consistent with the results shown in Table 3. Coho salmon (*On-*

TABLE 2. Difference in *Chondrococcus columnaris* exposure, infection, and antibody synthesis in largescale suckers from the North Shore Bonneville fish ladder and from a sampling site several miles downstream.

	N. Bonneville ladder	Downstream site
Sampling date	9/27/66	9/28/66
Temp (*C*)	18.9	18.5
No. fish sampled	20	40
% exposed[a] (not infected)	55	35
% infected[b]	40	5
Avg titers	1:500	1:200

[a] 1–10 *C. columnaris* external isolates/fish.

[b] >10 *C. columnaris* external isolates/fish or one or more *C. columnaris* internal isolate/fish.

TABLE 3. *Chondrococcus columnaris* infection and antibody development during upstream migration of coho salmon.

	Bonneville	Priest Rapids	Rocky Reach
Sampling date	9/27/66	10/5/66	10/18/66
Temp (*C*)	18.9	16.7	15.3
No. fish sampled	22	20	20
% infected[a]	5	20	45
Avg titers	1:38	1:61	1:232

[a] >10 external isolates/fish or one or more internal isolates/fish.

corhyncus kisutch) were sampled for disease during passage through the north shore Bonneville fish ladder, the Priest Rapids ladder, 200 miles and three dams upstream from Bonneville Dam and at the Rocky Reach ladder, 70 miles and two dams above Priest Rapids Dam, on the Columbia River. These fish were sampled at intervals of 8 and 21 days, respectively, after passage through the Bonneville ladder, and thus may roughly represent the same cohort of migrating fish. The experiment was designed to determine whether an increase in incidence of *C. columnaris* infection and antibody synthesis will occur during migration through fish ladders contaminated with high concentrations of organisms. Definite increases in infection and titer magnitude were observed for the salmon during upstream migration.

Some species of adult anadromous salmonids entering the Columbia River had low levels of *C. columnaris* antibodies, probably a residual titer from infection as juveniles (Fujihara 1970a). Although the disease is endemic in lower Columbia River coarse fishes, infected adult salmonids have not been observed until the fish reached Bonneville. The implication is that major infection and subsequent antibody synthesis occurs as a result of exposure to infected fish in the fish ladders.

Discussion

The narrow physical confines of fish ladders through which anadromous salmonids must pass during the warmwater months of the year make these sites a suspect area for *C. columnaris* exposure and infection. The fact that Columbia River water, other than from fish ladders, has been consistently negative for *C. columnaris*, makes it unlikely that a major infection will occur in the normally flowing portions of the river.

High rates of *C. columnaris* release in fish ladders, measured between June and September, occur at periods when fish occupancy in, and passage through, ladders was greatest. Since a single immune carrier yearling rainbow trout (*Salmo gairdneri*) can release up to 1×10^7 organisms/hour under laboratory conditions (Fujihara et al. 1964), and since crowded conditions are known to enhance disease problems (Pascha MS 1961), the release of large numbers of organisms by fishes in ladders is a reasonable interpretation of our data. Although the migrant salmon may move rapidly through areas of high *C. columnaris* density, the presence of such unusual concentrations may be sufficient for infection to occur.

The release of large numbers of *C. columnaris* is not a problem unique to fish ladders. The investigations of Colgrove and Wood (1966) estimated releases of *C. columnaris* organisms from the Horsefly River in the Fraser River system in Canada of nearly 3×10^9 cells/second. High temperatures on the spawning ground were believed to have been the major cause of this condition, although a heavy population density of sockeye salmon must have been a contributing cause. Similar calculations show releases of 1.7×10^6 to 3.3×10^8 *C. columnaris* cells/second from the Columbia River fish ladders.

The immune status of these salmon that develop moderately high agglutinating antibodies against *C. columnaris* disease during migration is not known and would be difficult to evaluate. Our laboratory studies, however, showed that juvenile coho salmon orally vaccinated against *C. columnaris* developed average agglutinating titers of 1:168 and were immune to the disease (Fujihara 1970b). It is conceivable that repeated exposure of migrating salmonids to *C. columnaris* may have some indirect benefit. Challenges to the disease at successive ladder facilities may induce antibody synthesis sufficient to protect the salmonids during migration.

Acknowledgments

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Effects of Jaw Tagging on Growth and Scale Characteristics of Landlocked Atlantic Salmon, *Salmo salar*

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WARNER, K. 1971. Effects of jaw tagging on growth and scale characteristics of landlocked Atlantic salmon, *Salmo salar*. J. Fish. Res. Bd. Canada 28: 537–542.

Recapture of 165 annual and biennial spawners of landlocked Atlantic salmon (*Salmo salar*) from 1239 fish tagged on the spawning grounds from 1953 to 1955 at the Fish River Lakes, Maine, showed that jaw-tagged females grew significantly slower than untagged females of identical ages and cycles. For most maturing male salmon, growth increments were not significantly reduced by jaw tagging. A tendency for decreased growth increments with increase in age was apparent for both tagged and untagged fish.

Recognizable annuli were formed on the scales of 77–100% of 97 male salmon and of 74–97% of 187 female salmon between tagging and recapture. A significantly smaller percentage of females (74%) than males (100%) at large 1 year and recaptured on the spawning grounds had formed a recognizable annulus, but there was no significant difference between females (86%) and males (91%) recaptured by anglers. The significantly lower annulus recognition for annual female spawners was attributed to their demonstrated slower growth rate and possibly resorption of minimal marginal scale growth.

Spawning checks were recognizable on the scales of 69–100% of 97 males and 49–78% of 187 females recaptured. Male salmon recaptured on the spawning grounds formed a significantly higher percentage of recognizable spawning checks than females (both annual and biennial spawners), but there were no significant differences between sexes for angler-recaptured fish. Lower reliability in spawning check recognition for females was attributed to less severe marginal resorption resulting in no spawning check being formed, or obliteration of previous spawning checks by resorption of the small amount of marginal scale increment made by some annually spawning females, or both.

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THIS study describes an attempt to evaluate possible effects of jaw tagging on growth of landlocked Atlantic salmon (*Salmo salar*) and to determine the recognizability of annuli and spawning checks on the scales of fish of known age and spawning history between tagging and recapture. A corollary purpose was to study possible reasons for lack of recognizability of annuli and spawning checks on some salmon scales. The study was conducted at the Fish River Lakes, Maine, 1953–57.

Several authors have described adverse effects of jaw tagging on growth (Rounsefell and Everhart 1953; DeRoche and Bond 1957; DeRoche 1963; Smith 1957; Youngs 1958) and condition (Schuck 1942; Cooper and Benson 1951; Pechacek 1956) of fishes, presumably through interference with normal feeding activity. There is also some evidence that recognizability of annuli may be reduced as a result of decreased growth rate caused by jaw tagging (Alvord 1954; DeRoche and Bond 1957). Inaccuracies in interpretation of spawning checks on the scales of salmon have also been discussed by some workers (Blair 1937, MS 1938; Cooper 1940; Leggett and Power 1969).

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Study Area

The Fish River Lakes are the major waters of the Fish River watershed, an important subdrainage of the Saint John River system in Aroostook County, Maine. Lakes in the chain are mostly oligotrophic, although two lakes are homothermous. Surface water temperatures seldom exceed 22 C for extended periods of time. The eight lakes range in size from 358.6 to 3010.6 ha, and in maximum depth from 6.1 to 50.3 m. The major spawning and nursery areas are located in the riverlike thoroughfares connecting the lakes.

Materials and Methods

From 1953 to 1955, 1239 salmon were tagged with monel metal mandible ring tags fastened around the left mandible. All salmon were anesthetized with urethane to facilitate handling and tagging. Total length measurements, weights, and scale samples were secured from all salmon at tagging and, when possible, at recapture. Further details on methods of capture are given by Warner (1959).

Scales were read from enlargements (45×) with a Bausch and Lomb microprojector. Scales of all fish secured both at tagging and at recapture were read at least twice, and scale characteristics and spawning marks were studied and noted.

Possible effects of jaw tagging and other factors on salmon growth were evaluated by comparing growth increments (total length) of tagged salmon with those of untagged salmon having identical spawning cycles and ages. Data for untagged salmon were from the same spawning area and the same years as for tagged fish. Annual growth increments were compared for salmon (both sexes) that had spawned only in 2 successive years. Two-year growth increments were compared for salmon (both sexes) that had spawned only in alternate years. Only spawning run fish from fall samples were used in growth comparisons, because measurements of fish reported by anglers were not considered precise enough for accurate growth evaluation, and these samples were taken throughout the season.

Annuli were determined by the usual criteria. Bands of closely spaced and broken circuli, associated with fall and winter slowdown of growth and abruptly circumscribed by widely spaced circuli associated with spring speedup of growth, were the best criteria for annulus recognition. Scales with little growth on the scale periphery since tagging presented considerable difficulty in interpretation. Havey (1959) and Warner and Fenderson (1963) have demonstrated the validity of annulus formation on scales of hatchery-reared landlocked Atlantic salmon as an indicator of age.

The study on formation of annuli as indicators of age of salmon following jaw tagging was based on comparison of scales collected at Cross Lake Thoroughfare from fall spawning runs at tagging (284 fish) with those of the same fish recaptured by anglers (109 fish) or on spawning runs 1 or 2 years after tagging (175 fish). Too few fish were taken 3 years after tagging to permit meaningful comparisons. For angler-recaptured salmon, only fish caught after June 1 were used, because annulus formation in salmon from these waters has been shown to be virtually completed by this date (Warner and Fenderson 1963).

A spawning check was considered to be formed when spring growth resumed and the new circuli cut sharply across the ends of partly resorbed circuli, in most instances leaving a translucent scar. The recognizability of spawning checks on scales of salmon previously jaw tagged was determined by comparing scales collected from the fall spawning runs at tagging with those of the same fish recaptured in spawning runs or by anglers 1 or 2 years after tagging. These data measured the reliability of using spawning checks as indicators of spawning history for fish with known spawning history subsequent to tagging. Only fish recaptured by anglers after June 1 were used.

Growth Increments

To test possible effects of jaw tagging on growth, mean total length increments were compared for 165 tagged and 204 untagged fish of similar ages and spawning cycles (Table 1). Growth analyses for both annual (1-year increments) and biennial (2-year increments) spawners were made by analysis of variance, and by Duncan's multiple test (Steele and Torrie 1960) for differences between individual mean length increments.

For annual spawners (sexes combined), 1-year increments for tagged fish were significantly less than for untagged fish ($P < 0.01$). A significant interaction was also demonstrated between sex and tagging ($P < 0.01$). Further tests (Duncan's) between mean 1-year increments of tagged and untagged males of three age-groups showed no significant differences, but tagged females (ages IV–V, V–VI, Fig. 1) showed significantly smaller mean annual growth increments than untagged females. This explains the significant interaction between sex and tagging.

For biennial spawners (sexes combined), 2-year increments for tagged fish were also significantly less than for untagged fish ($P < 0.01$) but, unlike the findings for annual spawners, there were no significant differences between all males combined and all females combined for either tagged or untagged salmon ($P > 0.05$). For female biennial spawners, mean 2-year increments (Fig. 1) were significantly less (Duncan's test, $P = 0.05$) for tagged than for untagged fish. Tagged males grew significantly slower than untagged males in one instance (age V–VII), but there was no difference in growth increments between tagged and untagged males in two other comparisons (age III–V, age IV–VI, Fig. 1).

There was a definite tendency in both annual and biennial spawners for growth increments of both tagged and untagged fish (both sexes) to decrease with age (Fig. 1). For female annual spawners, mean increments of adjacent age-groups in older fish of similar tagging status differed significantly in three comparisons. But no significant differences were found for female biennial spawners or male annual or biennial spawners.

Sex appeared to be the most important factor governing the effect of jaw tagging on salmon growth. The data demonstrate that jaw tagging significantly reduced growth increments in female salmon for both annual and biennial spawners. Jaw tagging apparently had no significant effect on growth of annual male spawners and only a slight effect (one significant comparison) on biennial male spawners.

TABLE 1. Mean total lengths for various age-groups of tagged landlocked Atlantic salmon according to spawning periodicity in comparison with those for untagged fish. Number of fish in parentheses.

	Age													
Sex	III[a]		IV	IV		V	V		VI	VI		VII	VII		VIII
						Annual spawners									
							Tagged								
Females	–		–	474	(11)	496	561	(13)	576	636	(10)	651	625	(3)	632
Males	379	(9)	461	452	(7)	509	509	(5)	545	–		–	–		–
							Untagged								
Females	421	(9)	517	491	(29)	552	528	(17)	626	–		–	–		–
Males	399	(26)	477	502	(21)	561	552	(5)	606	–		–	–		–
	Age														
Sex	III		V	IV		VI	V		VII	VI		VIII	VII		IX
						Biennial spawners									
							Tagged								
Females	–		–	483	(34)	571	535	(22)	604	636	(11)	676	615	(3)	658
Males	415	(11)	535	487	(16)	595	535	(10)	625	–		–	–		–
							Untagged								
Females	416	(16)	583	491	(30)	630	536	(12)	645	–		–	–		–
Males	407	(11)	569	502	(20)	628	561	(8)	702	–		–	–		–

[a] The first age for each pair of values is the age at tagging or the comparable age for untagged fish, and the second the age at recapture or comparable age for untagged fish.

That jaw tagging may have a significant effect on fish growth or condition has been indicated by several workers. Growth retardation as a result of jaw tagging has been shown by DeRoche and Bond (1957) and DeRoche (1963) for lake trout (*Salvelinus namaycush*) and Smith (1957) for brook trout (*Salvelinus fontinalis*). Youngs (1958) studied the effects of mandible ring tags on five species of fish, including landlocked Atlantic salmon, and found that length increments of tagged and untagged fish were significantly different in 7 of 10 instances. Schuck (1942) compared the condition of tagged and untagged wild brown trout (*Salmo trutta*) in a New York stream and showed that tagging had a significantly negative effect on trout condition. Other studies have shown that jaw tagging adversely affected condition of cold-water fishes (Cooper and Benson 1951; Pechacek 1956).

Scale Characteristics

ANNULUS FORMATION

Two studies for Maine lakes have shown that ages can be determined from salmon scales with a fairly high degree of reliability. For known-aged hatchery-reared salmon from wild parents, ages were determined accurately in 80% (Havey 1959) and 87% (Warner and Fenderson 1963) of the samples. The purpose of the present study was to evaluate recognizability of annuli formed on the scales of wild annual and biennial spawners between

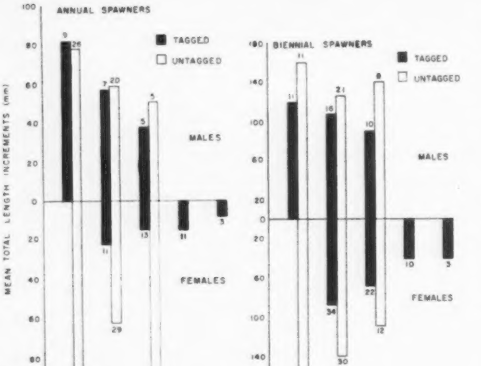

FIG. 1. Annual and 2-year growth increments (total length, mm) of tagged and untagged landlocked Atlantic salmon of each sex.

tagging and recovery and to study possible influences of sex, amount of growth, and spawning periodicity on recognizable annulus formation.

The null hypothesis that there were no differences in recognizable annulus formation between sexes and, where sample size was adequate, between fish that had grown more than and less than 25.4 mm after tagging was tested by χ^2 criteria.

Recognizable annuli were formed on the scales of 77–100% of 97 males and 74–97% of 187 females studied (Table 2). These data are consistent with previous findings for hatchery-reared salmon in Maine waters (Havey 1959; Warner and Fenderson 1963).

TABLE 2. Incidence of recognizable annuli and spawning checks in jaw-tagged landlocked Atlantic salmon recaptured in spawning runs and by anglers.

Comparison	Years at large	No. fish	Annulus formation (%)	Spawning check formation (%)
		In spawning run		
Males	1	23	100[a]	100[b]
Females		35	74	49
Males	2	40	83	98[b]
Females		77	90	75
		By anglers		
Males	1	21	95	86
Females		43	86	74
Males	2	13	77	69
Females		32	97	78

[a]Significant at $P = 0.05$.
[b]Significant at $P = 0.01$.

All (100%) male fish that had been at large 1 year after tagging and then recaptured on spawning grounds had formed a recognizable annulus (Table 2). However, only 74% of females at large 1 year and then recaptured had formed an observable annulus. Male salmon formed a significantly higher percentage of recognizable annuli than females ($P < 0.05$). For angler-recaptured fish at large 1 year, however, there was no significant difference in percentage of recognizable annulus formation between males (95%) and females (86%).

Because other studies (Alvord 1954) have shown a relation between amount of growth after tagging and annulus formation (perhaps more appropriately stated annulus recognition), a χ^2 test was run in the present study (in the only instance where sample size was adequate) to determine any significant difference in annulus recognition between females that had grown more than 25.4 mm and those that had grown less after tagging. Although the difference attributable to growth was not significant ($P > 0.05$) in this instance, there was a tendency for females that had grown more than 25.4 mm after tagging to exhibit a higher percentage of recognizable annuli than those with less growth. Alvord (1954) found that failure to form annuli by jaw-tagged brown trout, rainbow trout (*Salmo gairdneri*), and brook trout was almost always accompanied by little or no increase in total length, especially in older fish. For fish with less than 0.8 inches (20.3 mm) growth in total length, an expected new annulus was lacking in 59.4% of the brown trout and 33.3% of the rainbow trout. DeRoche and Bond (1957) found that some jaw-tagged lake trout failed to form annuli in 1- and 2-year periods at large. Failure to form annuli by tagged fish was associated with less-than-normal growth rate.

No significant differences in percentage of recognizable annulus formation were found between males and females that had been at large 2 years and recaptured either by anglers or on the spawning grounds (Table 2). Only scales on which two annuli were recognizable were counted as valid interpretations.

These data indicate a fairly high degree of reliability in interpretation of annuli in jaw-tagged landlocked salmon. The significantly lower percentage of annulus recognition only from the scales of female salmon returning to the spawning grounds after 1 year at large is probably related to the previously demonstrated slower growth of these tagged annual spawners. If indeed an annulus were formed, it may have been obliterated by only slight marginal resorption. It is possible also that an annulus was present but unrecognizable because of abnormal growth pattern of circuli.

SPAWNING CHECK FORMATION

The purpose of this part of the study was to determine incidence of single recognizable spawning checks, formed between tagging and recovery, on the scales of annual or biennial spawners with known spawning history. A corollary objective was to study any influence of sex, amount of growth, and spawning periodicity on spawning check formation or recognizability.

A single spawning check was observed on the scales of 69–100% of all males and 49–78% of all females studied (Table 2). Spawning check recognition on the scales of jaw-tagged salmon was tested using X^2 criteria. The null hypothesis was that there were no differences in spawning check formation between sexes or, where sample size was adequate, between fish that had grown more than 25.4 mm after tagging and those that had grown less.

All (100%) of the tagged males recaptured on the spawning run after being at large 1 year had formed a recognizable spawning check. Female salmon, however, had formed a significantly ($P < 0.01$) smaller percentage (49%) of recognizable checks. Females that had grown less than 25.4 mm after tagging showed a smaller percentage of check formation (37%) than those that had grown more than 25.4 mm (73%), but X^2 tests showed that this difference was significant at only $\beta = 0.20$. For angler-recaptured fish at large 1 year, there was no significant difference in percentage of check formation between males and females ($P > 0.05$), and there were no differences attributable to amount of growth after tagging for females. The sample size was not adequate to make valid comparisons for males.

For fish recaptured in the spawning run the 2nd year after tagging, and with a known alternate-year spawning history, 98% of the males and 75% of the females had formed spawning checks. As with annual spawners, males spawning in alternate years formed a significantly higher percentage of checks than females ($P < .01$).

For salmon that had been at large 2 years and were recaptured by anglers, no significant difference was found in percentage of males (69%) and females (78%) that had formed spawning checks.

The data for these waters show that a single spawning check was interpreted fairly accurately from the scales of jaw-tagged male salmon whether they had spawned annually or in alternate years. Other workers and observations in the present study showed that male salmon usually form a much more well-defined spawning check than females. This is attributed to a more marked resorption of the scale margin in males, usually followed by considerable scale growth and repair (Menzies 1925; Blair MS 1938; Cooper 1940). Although not included in the above summary, spawning history could usually not be interpreted accurately from the scales of male salmon from these lakes that had spawned more than twice. Severe marginal and surface resorption commonly obliterated one or more spawning checks. In severe instances, scales were completely unreadable. Cooper (1940) found that scale resorption in salmon from Rangeley Lakes, Maine, that had spawned several times often completely obliterated the history of even the first spawning.

The lower percentage of spawning check formation in female salmon appeared to be associated with two factors. First, resorption of the scale periphery was less severe in females than in males. Even though recognizable annuli had been formed in some instances, associated spawning marks were apparently not formed. Secondly, because many females at large 1 year had grown less than 25.4 mm after tagging, growth of the scale margin was very slight, and even a small amount of resorption in the "shoulder" region of the scale could obliterate a previously formed, ill-defined spawning check. For recaptures by anglers, spawning checks could be interpreted as reliably for females as for males (Table 2). This supports the observation that previous spawning checks may be obliterated by resorption of the small amount of marginal scale growth made by some annually maturing females. White and Medcof (1968) found that the spawning history of a female sea-run Atlantic salmon with a known spawning history could not be determined accurately from spawning checks when the salmon had spawned two or more times. Blair (1937) found that severe marginal resorption associated with annual spawning resulted in inaccuracies in interpreting scales of landlocked Atlantic salmon. Leggett and Power (1969) found that mature landlocked Atlantic salmon in Flatwater and Gambo Ponds, Nfld., frequently did not form spawning checks. These authors attributed lack of spawning check formation to lack of scale resorption at spawning in some fish.

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A Key to the Freshwater Hirudinoidea of Canada

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A key to the freshwater leeches (Hirudinoidea) of Canada and the bordering states of the USA is presented, together with notes on their important diagnostic features.

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THE leech fauna has generally been overlooked or ignored by limnologists in Canada. In part this may be due to the absence of a suitable key or to the scant and widely scattered literature, which is often difficult to interpret and assess. The key presented here is intended to alleviate the first problem and a later paper (in preparation) will deal with the distributions of species in Canada. Because of the larger volume of work carried out in the United States of America, this key also includes species that have as yet not been recorded in Canada but have been recorded in one or more of the bordering states. These include *Actinobdella inequiannulata*, *Illinobdella richardsoni*, *Mooreobdella bucera*, *Percymoorensis lateromaculata*, *Philobdella gracile*, *Piscicolaria reducta*, and *Placobdella pediculata* (marked with an asterisk (*) in the key). On the basis of an identification made by J. P. Moore in Rawson (1953), *Erpobdella octoculata* is also included despite statements by Mann (1961) and by Soós (1968) that this species is restricted to the Palearctic region.

Until now the only keys available have been those of Moore (1918, 1959) for the whole of North America, that of Pennak (1953) for the United States, and the combined key to the freshwater leeches of central Europe, the British Isles, and North America north of the Rio Grande by Mann (1961). These keys are unnecessarily complex for workers in Canada and the bordering states of the USA because of the inclusion of many species not found in the area, and inaccurate because of the ommission of several species that should be included. Regional keys have been written by Moore (1906, 1922) for the Great Lakes region and southern Canada, by Bere (1929) for Jasper Park Lakes (Alberta), and by Moore (1964, 1966) for Alberta. However, even when written, these keys were of little use outside the designated areas and, due to the limited collections upon which they were based, are now of little value within the areas. Furthermore both the continental and regional keys require revision in the light of the recent taxonomic changes proposed by Richardson (1969) and Soós (1963, 1965, 1966, 1969a, b).

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Notes on Use of the Key

Identification of leeches is extremely difficult and often impossible with poorly preserved specimens. It is assumed for use of this key that either living or well-preserved material is available. Live leeches can be narcotized in soda water and then, if required, preserved in either 70% alcohol or 4% formaldehyde. An alternative method is that favoured by Mann (1961): 70% alcohol is dripped into the water containing the leeches gradually over a period of 30 min until the leeches no longer move.

External features such as the following are used whenever possible: annulation; eye position and number; presence or absence of papillae, tubercules, and pulsatile versicles; relative size of suckers; position and size of mouth; and positions of male and female genital pores. Colour differences are generally ignored because of the high degree of variability within species and the frequent reduction and alternation of coloration after preservation. The only notes on coloration included are those known to persist for several years after preservation. In a few instances reference is made to internal anatomical features, the observation of which necessitates dissection. If reference is made to such a feature in a couplet after reference to an external feature, the key can be used without resorting to the dissection provided the external features can be clearly seen. The only obligatory dissections are: examination of the atrial cornua to distinguish *Nephelopsis obscura* from the *Dina-Mooreobdella* genera complex, and examination of the jaws and teeth for the identification of some Hirudinidae.

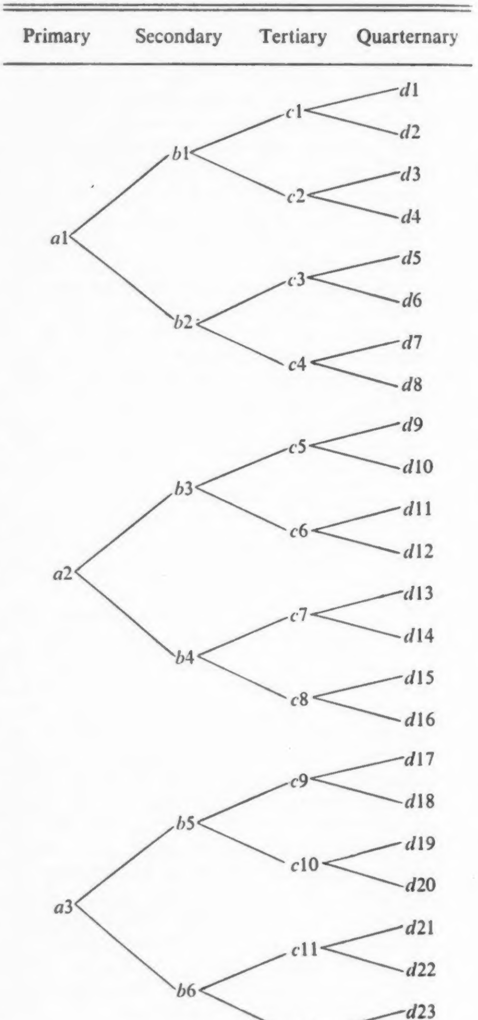
The body of a leech consists of 34 somites (designated I through XXXIV), each somite being represented by a ganglion in the central nervous system. Each somite is usually divided externally by superficial furrows into 2–16 annuli. Somites with the full number of annuli (termed *complete somites*) are found in the middle of the body and this number is generally characteristic of the genus or species. Incomplete somites occur at both ends of the body. Reference to annulation in the key is always to complete somites. Annuli features can be most easily seen in the lateral margins of the ventral surface.

Moore (1898) recognized that the nerve cord ganglia are placed in the middle annulus of the somite and that the triannulate somite is the basic form. In Moore's suggested nomenclature, which is used throughout this key, the three primary annuli are numbered *a*1, *a*2, and *a*3, from the anterior. Annulus *a*2 (the *neural annulus*) contains the ganglion and is marked externally by transverse rows of cutaneous sensillae (*segmental receptors*). Repeated bisection of these primary annuli give more complex annulation (Table 1).

In addition to the sensillae, other surface organs are the eyes, papillae, and tubercules. When present on the posterior sucker, the eye spots, which are embedded in pigment, are termed *oculiform spots*. Oculiform spots are commonly present in the Piscicolidae but are absent in the Glossiphoniidae. The number and position of the eyes are important diagnostic features. The Glossiphoniidae have one to four pairs of eyes, the Piscicolidae zero to two pairs, Erpobdellidae three or four pairs (except *Dina anoculata*) and the Hirudinidae five pairs. In *Batracobdella* and *Glossiphonia* coalescence of eyes sometimes occurs, the lobed nature of the eyes usually indicating the original condition. The relative distance between eyes is often important in identification. If the distance between a pair of eyes is less than the diameter of a single eye they are termed *close*; if the distance between them is equal to or greater than this they are termed *well separated.* In the Erpobdellidae the eyes are arranged in separate labial and buccal groups, whereas in the Hirudinidae the eyes are arranged in an arc around the anterior margin (Fig. 3).

Papillae are small protrusible sense organs that are often widely scattered over the dorsal surface. *Tubercules* are larger protrusions that include some of the dermal tissues and muscles, and are themselves sometimes covered with papillae. The disposition of the papillae and tubercules is important in distinguishing the species of *Placobdella*. In *Placobdella ornata* tubercules are present and the surface of the tubercules is covered with papillae, giving them a rough or warty appearance. On the other hand *Placobdella parasitica* and *P. papillifera* do not have tubercules and the papillae, when present, are smooth rounded cones.

TABLE 1. Theoretical table of the annuli produced by repeated bisection of the three primary annuli (after Moore 1898).



In the Piscicolidae (excepting *Illinobdella* and *Piscicolaria*) the body is divided into two regions: a narrow anterior region, the *trachelosome*, and a longer and wider posterior region, the *urosome*. In *Piscicola* and *Cystobranchus* only, the neural annuli of the urosome bear paired pulsatile vesicles. In *Cystobranchus* the vesicles are large and clearly visible in both living and preserved material but in *Piscicola* (Fig. 1) they are small and sometimes invisible in preserved specimens.

The anterior sucker may be very prominent or may simply consist of the expanded lips of the mouth. In the Piscicolidae the anterior sucker is always expanded and generally forms a conspicuous dilation on the head, whereas in the Glossiphoniidae it is generally small and never expanded. The posterior sucker is generally directed ventrally. Except for *Myzobdella*, *Illinobdella*, and *Piscicolaria*, all the Piscicolidae have the posterior sucker markedly wider than the body at the point of attachment.

The alimentary canal is divisible into the buccal chamber, pharynx, oesophagus, crop, intestine, and rectum. In the Rhynchobdellae the mouth is a small pore either on the edge or in the center of the anterior sucker (Fig. 7). The pharynx is muscular and protrusible through the mouth as a proboscis. In the Arhynchobdellae the mouth is large and occupies the cavity of the anterior sucker. In the Hirudinidae the buccal cavity, which may or may not contain jaws, is separated from the cavity of the sucker by a flap of skin, the *velum* (Fig. 11). The presence or absence of papillae on the velum is the key distinguishing character between *Bdellarogatis plumbeus* (papillate) and *Mollibdella grandis* (smooth). With the exception of these two species all Hirudinidae have three muscular jaws (two ventrolateral, one dorsomedial), the free edge of each bearing teeth arranged in one (*monostichodont*) or in two (*distichodont*) rows (Fig. 12).

For examination of the velum and jaws the specimen should be pinned out and a median ventral incision made from the lower lip of the anterior sucker back far enough for the margins to be pinned out to expose the inner surface of the pharynx. Details of the teeth can only be seen by removal of a jaw and making a mount on a microscope slide.

The anus opens on the dorsal surface just anterior to the posterior sucker except in *Placobdella pediculata* and *Illinobdella richardsoni*. In *I. richardsoni* the anus opens 15 annuli anterior to the sucker, and in *P. pediculata* it opens between somites XXIII and XXIV with the posterior annuli forming a slender stalk (peduncle) bearing the posterior sucker.

The gonopores are visible on the midline of the ventral surface of somites XI and XII and are generally separated by two to five annuli; the male gonopore is anterior to the female gonopore and is larger and more readily visible. *Marvinmeyeria lucida* and *Glossiphonia heteroclita* are unusual in that the male and female ducts open into a common gonopore.

The female reproductive system consists of a pair of ovisacs, the end of each ovisac terminating in a tube, which dilates to form the vagina.

The male reproductive system consists of testisacs arranged in metameric pairs, except in the Erpobdellidae in which they are arranged in grapelike clusters. On each side of the body the vasa efferentia run into the vas deferens; this duct leads into the seminal vesicle and the ejaculatory duct, which opens into the atrium. The atrium is a median chamber with a pair of lateral horns (atrial cornua), which receive the ejaculatory ducts. In *Nephelopsis obscura* the atrial cornua are spirally arranged like rams' horns, but in the *Dina-Mooreobdella* complex the cornua are simply curved (Fig. 9). Confirmation of this feature necessitates dissection. The preserved specimen should be pinned out with the ventral surface up and a transverse incision made across the body three or four annuli posterior to the male gonopore. Cuts should then be made anteriorly up the lateral margins of the body for about 20 annuli. The posterior edge of the flap can now be lifted forward, exposing the inner tissues, which can be cleared away to fully expose the atrium. Mann (1961) prefers to cut a rectangular portion of the body wall containing the atrium and examine it separately. The disadvantage of this method is that if the piece of body wall is lost the specimen from which it was removed is thereafter unidentifiable.

Soós (1968) considers that *Dina* s.str. and *Mooreobdella* are subgenera of the genus *Dina*. Moore (1959) considers them as distinct genera. In *Dina* s.str. the vasa deferentia form preatrial loops, which extend to ganglion XI, whereas in *Mooreobdella* they fail to produce preatrial loops. Determination of this point is difficult and time consuming, and in the key genera (or subgenera) have been lumped into the *Dina-Mooreobdella* complex, which can be separated on external features.

With the exception of three triplets (each marked *a*, *b*, and *c*), dichotomous couplets are used throughout the key.

Key

1. Mouth a small pore in anterior sucker through which a muscular pharyngeal proboscis may be protruded. No jaws or denticles. Order Rhynchobdellae..............................2

Mouth large, occupying entire cavity of anterior sucker. No proboscis. Jaws either present or absent. Order Arhychobdellae..3

2. Anterior sucker expanded and distinct from body. 0–2 pairs of eyes. Body cylindrical; usually long and narrow with, at rest, a length-to-width ratio of more than 10:1 (exceptions: *Cystobranchus*, *Myzobdella*, *Piscicola punctata*, *Piscicola salmositica*, and *Piscicoloria*, for which ratio is 6 or less to 1). In *Illinobdella* and *Piscicolaria* only, body divided into narrow anterior (trachelosome) and wider posterior (urosome) regions. Oculiform spots sometimes present on posterior sucker. Pulsatile vesicles present along lateral margins of urosome in *Piscicola* and *Cystobranchus*. All with 7 or more annuli per somite except for *Piscicolaria reducta*, which is triannulate. Neither eggs nor young attached to ventral surface of adult during development. Family Piscicolidae. Fig. 1, 4, 5..........4

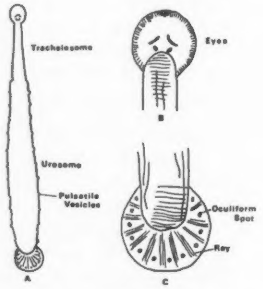


FIG. 1. *Piscicola geometra*: A, expanded anterior and posterior suckers, 11 pairs of small pulsatile vesicles, and division of the body into trachelosome and urosome; B, anterior sucker showing relative size and position of eyes; C, posterior sucker showing oculiform spots and dark rays. All dorsal views.

Anterior sucker more or less fused to head and always narrower than the head. (*Placobdella montifera* has an expanded discoid head that might be mistaken for a piscicolid feature.) Body flattened and never cylindrical (except for *Helobdella elongata*, which is subcylindrical). At rest, body with a length-to-width ratio of less than 10:1. Body never divided into trachelosome and urosome. No oculiform spots on posterior sucker. Somites triannulate. Eggs and young attached to ventral surface of adults during early development. Family Glossiphoniidae. Fig. 2...............7

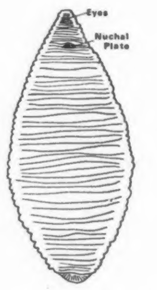


FIG. 2. Dorsal view of *Helobdella stagnalis* showing general body shape, nuchal plate, and number and position of eyes.

3. 3–4 pairs of eyes (absent in *Dina anoculata*), in separate labial and buccal groups. No jaws. Pharynx about one-third of body length. Testes small and very numerous. Family Erpobdellidae. Fig. 3.............................13

5 pairs of eyes, arranged in an arch on somites II–VI with 3rd and 4th pairs separated by 1 annulus. Jaws either present or absent. Pharynx short. 9 or 10 pairs of testes arranged metamerically. Family Hirudinidae. Fig. 3............15

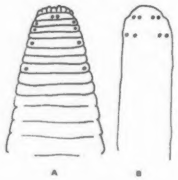


FIG. 3. A, anterior of *Macrobdella decora* showing arrangement of the five pairs of eyes; B, anterior of *Dina parva* showing the four pairs of eyes in labial and buccal groups. Both dorsal views.

4. Posterior sucker appreciably wider than body at point of attachment. Pulsatile vesicles on lateral margins of neural annuli of urosome. 1 or 2 pairs of eyes. 5

No pulsatile vesicles on urosome. Posterior sucker narrower than body. (In *Illinobdella* sp. the posterior sucker is occasionally the same width as the body.) 0–1 pair of eyes. 6

5. 11 pairs of large and distinct pulsatile vesicles (easily seen in both preserved and live specimens). Trachelosome and urosome both sharply distinct and flattened dorsoventrally. Both suckers large, the posterior sucker being about 2½ times the diameter of anterior sucker. 2 pairs of eyes, the 1st pair forming conspicuous dashes at 45° to longitudinal axis; the 2nd pair are ovoid. No oculiform spots on posterior sucker. Length-to-width ratio 6:1. Gonopores separated by 2 annuli. Somites 7 annulate. . . . . *Cystobranchus verrilli* Meyer, 1940

11 pairs of pulsatile vesicles not very apparent in preserved material. Trachelosome and urosome not sharply distinct. Body cylindrical or slightly flattened. Both suckers wider than body at point of attachment, the posterior sucker being about twice the diameter of the anterior. 2 pairs of eyes (rarely 1 pair). Oculiform spots present on posterior sucker except in *Piscicola punctata*. Length-to-width ratio 10 or more to 1 (except in *P. punctata* and *Piscicola salmositica*). Somites 14 annulate. Genus *Piscicola*. Fig. 1. 20

6. (*a*) Body divided into a small trachelosome and a larger urosome. Length-to-width ratio 3–6:1. 1 pair of eyes. Somites 14 annulate. Posterior sucker a concavity of the posterior body. Fig. 4. . . . .*Myzobdella moorei* (Meyer, 1940)

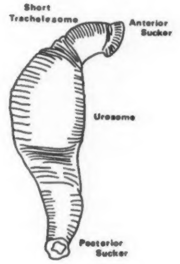


FIG. 4. *Myzobdella moorei* showing body divided into a short narrow trachelosome and longer wider urosome; and relative size of suckers.

(*b*) Body not divided into trachelosome and urosome. Length-to-width ratio 10 or more to 1. 0–1 pair of eyes in posterior half of head. Somite either 12 or 14 annulate. Genus *Illinobdella*. Fig. 5. 24

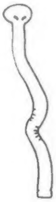


FIG. 5. *Illinobdella alba* showing body undivided into regions, and relative size of the suckers.

(*c*) Body not divided into trachelsome and urosome. Length-to-width ratio 4 or 5:1. 1 pair of eyes. Somites triannulate. . . . . *Piscicolaria reducta* Meyer, 1940*

7. Posterior sucker conspicuous with a marginal circle of about 30 or 60 glands and retractile papillae, their positions being indicated dorsally by faint radiating ridges. Genus *Actinobdella*. Fig. 6. 26

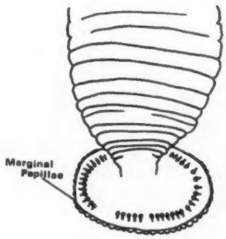


FIG. 6. Dorsal view of posterior sucker of *Actinobdella annectens* showing marginal papillae, and dorsal ridges.

Posterior sucker without a marginal circle of glands and retractile papillae. 8

8. 0–2 pairs of eyes 9
   3–4 pairs of eyes 12

9. Mouth apical or subapical on rim of the anterior sucker. Fig. 7A 10
   Mouth within anterior sucker and clearly not on rim. Fig. 7B 11

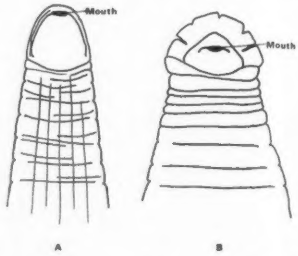


FIG. 7. A, anterior sucker of *Marvinmeyeria lucida* showing apically placed mouth on the rim of the sucker; B, anterior sucker of *Helobdella stangalis* showing mouth situated in the center of sucker. Both ventral views.

10. Male and female gonopores united in a common bursal pore. 1 pair of eyes well separated (1–2 times the diameter of a single eye). Body smooth, without papillae. Lateral margins of body very thin and translucent .... *Marvinmeyeria lucida* (Moore, 1954)
    Male and female gonopores separated by 2 annuli. 0–1 pair of eyes (except *P. hollensis*, which has several pairs of accessory eyes on neck). Eyes close together or confluent (except *P. montifera*, which has eyes well separated). Body usually papillate. Lateral margins thin but not translucent. Genus *Placobdella* 28

11. 1 or 2 pairs of eyes. If only 1 pair of eyes present, these close together or lobed, indicating that they have coalesced. Gonopores separated by 2 annuli. Genus *Batracobdella* 34
    1 pair of eyes, well separated. Gonopores separated by 1 annulus. Segmentation conspicuous. Genus *Helobdella* 35

12. 4 pairs of eyes, on paramedian lines of somites II–V. Body very soft. Genus *Theromyzon* 37
    3 pairs of eyes (coalesced eyes sometimes occur, their lobed nature indicating the original condition). Body firm. Genus *Glossiphonia* 39

13. Somites 5 annulate (*b*1, *b*2, *a*2, *b*5, and *b*6, with *b*6 never distinctly subdivided), all annuli being equal in length. Genus *Erpobdella*. Fig. 8B 40
    Somites 6 or 7 annulate, annuli differing in length and *b*6 either subdivided or longer than the others (in any group of 6 consecutive annuli at least 1 is either narrower or wider than the rest, the wider or narrower annulus occurring regularly along the body). 3 or 4 pairs of eyes. Fig. 8A 14

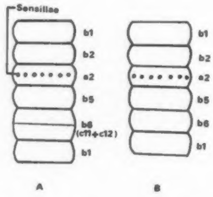


FIG. 8. A, the annulation of *Dina-Nephelopsis* showing subdivision of *b*6; B, *Erpobdella* showing the undivided nature of annulus *b*6.

14. Atrial cornua spirally coiled like a ram's horn. Dorsum dotted with black spots, the edges of which finely branch and ramify, usually with anastomosing terminal branches. 4 pairs of eyes (2 labial and 2 buccal). Fig. 9A .... *Nephelopsis obscura* Verrill, 1872
    Atrial cornua simply curved. Dorsum without spots having anastomosing terminal branches. 3 or 4 pairs of eyes (rarely none). Genera *Dina*: *Mooreobdella*. Fig. 9B 41

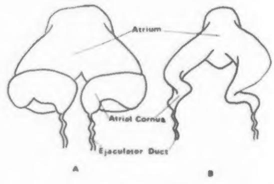


FIG. 9. A, the spirally curved atrial cornua of *Nephelopsis obscura*; B, the simply curved atrial cornua of *Dina parva*.

15. Copulatory glands behind the gonopores. Fig. 10..........16

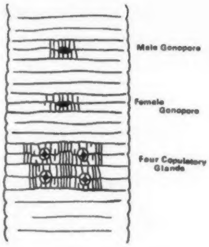


FIG. 10. Ventral view of gonopores of *Macrobdella decora* showing arrangement of the surrounding copulatory glands.

Copulatory glands absent..........17

16. 4 large copulatory pores behind gonopores, which are separated by 5 annuli. Dorsum with median longitudinal line of red spots, which appear yellowish-white after preservation, and a line of dark spots down each margin. Jaws well developed with about 65 monostichodont acute teeth. 10 pairs of testes. Fig. 10, 11..*Macrobdella decora* (Say, 1824)

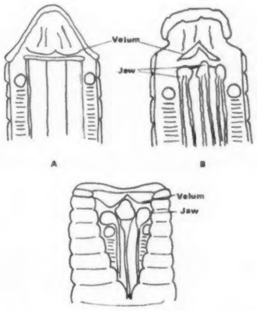


FIG. 11. Ventral view of the dissection of mouth and buccal cavity of: (A) *Mollibdella grandis*, (B) *Percymoorensis marmorata*, (C) *Macrobdella decora*, to show velum, relative sizes of jaws of *M. decora* and *P. marmorata*, and absence of jaws in *M. grandis*.

Copulatory gland pores in 2 nearly equal groups associated with the 2 gonopores, which are in the glandular area. Gonopores separated by 3–4 annuli but obscured in mature leeches by the copulatory pits and prominences. Jaws well developed with 35–45 teeth, wholly or partially distochodont. 7 pairs of testes..........*Philobdella gracile* Moore, 1901*

17. Jaws absent. Fig. 11A..........18
Jaws present and minutely denticulate..........19

18. Lower surface of the velum smooth. Gonopores in the furrows between the annuli and separated by 5 annuli. Pharynx with 12 internal ridges..........*Mollibdella grandis* (Verrill, 1874)
Lower surface of the velum finely and closely papillate. Gonopores in the middle of the annuli, separated by 5½ annuli. Pharynx with 15 internal ridges....*Bdellarogatis plumbeus* (Moore, 1912)

19. Jaws very small and retractable into narrow-mouthed tubular pits. 9–25 coarse distochodont teeth per jaw. Genus *Percymoorensis*. Fig. 11B, 12..........44
Jaws very large with 35–100 acute monostichodont teeth. Fig. 12B..........*Hirudo medicinalis* Linn., 1758

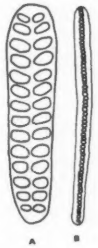


FIG. 12. A, jaw of *Percymoorensis marmorata* showing distichodont arrangement of teeth; B, jaw of *Macrobdella decora* showing monostichodont arrangement of teeth.

20. Length-to-width ratio 6 or less to 1. Somites 3 or 14 annulate. Oculiform spots either present or absent..........21
Length-to-width ratio 10 or more to 1. Oculiform spots present. Somites 14 annulate..........22

21. 8–10 cresent-shaped oculiform spots on the posterior sucker. Somites 14 annulate..........*Piscicola salmostica* Meyer, 1946
Oculiform spots absent. Triannulate..........*Piscicola punctata* (Verrill, 1871)

22. Posterior sucker with 10–12 oculiform spots and no dark rays. Anterior pair of eyes like heavy dashes twice as long as wide................................*Piscicola milneri* (Verrill, 1874)
Posterior sucker with dark rays and 12–14 oculiform spots................................23

23. Posterior sucker with 12–14 oculiform spots separated by an equal number of dark rays. Anterior pair of eyes like fine dashes 5 times as long as wide. Fig. 1B......*Piscicola geometra* (Linn., 1758)
Posterior sucker with 14 dark rays, each terminating in an oculiform spot...*Piscicola zebra* Moore, 1898

24. Length-to-width ratio about 10:1. Posterior sucker occasionally as wide as adjacent body area but usually smaller. Somites 14 annulate. Fig. 5...................*Illinobdella alba* Meyer, 1940
Length-to-width ratio 15 or more to 1..25

25. Length-to-width ratio 15:1. Posterior sucker little more than a concavity of the posterior body. 1 pair of eyes (sometimes absent). Anus 15 annuli anterior to posterior sucker. Somites 14 annulate..*Illinobdella richardsoni* Meyer, 1940*
Length-to-width ratio 18:1. Posterior sucker half as wide as body. 1 pair of eyes. Anus 10 annuli or less anterior to sucker. Somites 12 annulate.............*Illinobdella elongata* Meyer, 1940

26. Posterior sucker with a marginal circle of about 60 glands and retractile processes. Somites 6 annulate with *b*3 and *b*5 the largest and most conspicuous annuli. Dorsal papillae in 5 longitudinal series, the median series on *b*3 and *b*5 being the most prominent..*Actinobdella annectens* Moore, 1906
Posterior sucker with a marginal series of about 30 glands and retractile processes. Somites either 3 or 6 annulate. Either 1 or 5 series of dorsal papillae.................................27

27. Somites 3 annulate with *a*2 larger than either *a*1 or *a*2. Dorsal papillae in 5 series on *a*2 and *a*3...*Actinobdella triannulata* Moore, 1924
Somites 6 annulate. Annuli of unequal size with *b*3 and *b*5 the largest. Papillae on *b*3 and *b*5 in 1 median series................................*Actinobdella inequiannulata* Moore, 1901*

28. 1 pair of eyes on somite III. No supplementary eyes....................................29
Pair of eyes on somite III followed by an indefinite number of pairs of supplementary eyes. First 3 pairs of eyes most prominent. Cutaneous tubercules large and rough..*Placobdella hollensis* Whitman, 1892

29. Anus between somites XXIII and XXIV with the postanal annuli (usually 16) forming a slender stalk (peduncle), which bears the posterior sucker. Peduncle not formed in specimens less than 1 cm long. No cutaneous papillae...................*Placobdella pediculata* Hemingway, 1908*
Anus close to the posterior sucker. No peduncle..30

30. Margins of posterior sucker denticulate. Fig. 13..........31

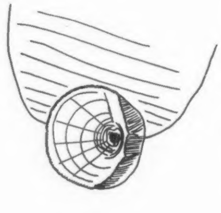


FIG. 13. Posterior sucker of *Placobdella montifera* showing denticulate margin.

Margins of posterior sucker not denticulate...32

31. Head expanded and discoid. Dorsum with three low dorsal keels, each bearing large conical papillae. Eyes separated by about their own diameters.............*Placobdella montifera* Moore, 1906
Head not discoid; dorsal keels absent. Eyes close together or confluent. Prominent yellow or white band on somite VI.................................*Placobdella phalera* (Graf, 1899)

32. Median longitudinal row of tubercules especially conspicuous. All tubercules bearing several papillae, which impart a rough warty appearance.................... *Placobdella ornata* (Verrill, 1872)

Dorsal papillae, when present, simple cones with smooth domes. Either conspicuous or inconspicuous 33

33. Dorsal papillae either inconspicuous or absent. When present, arranged in 5 longitudinal rows. Ventral surface with 11–12 blue, brown, or green stripes. Annulus *a*3 without cross furrow.... .... *Placobdella parasitica* (Say, 1824)

Dorsal papillae small and conical but distinct. Ventrum without longitudinal stripes. Annulus *a*3 of each somite in the middle of the body with distinct cross furrow.................... .... *Placobdella papillifera* (Verrill, 1872)

34. A small pair of eyes on somite III and a larger pair on IV. Eyes sometimes coalesced. Papillae absent.. *Batracobdella paludosa* (Carena, 1824)

1 pair of eyes on somite III only, sometimes confluent. Low small papillae present........... .... *Batracobdella picta* (Verrill, 1872)

35. Horny scute (nuchal plate) on the dorsum of somite VIII about one-sixth of the way between the anterior and posterior suckers. Fig. 2................ *Helobdella stagnalis* (Linn. 1758)

Nuchal plate absent..36

36. Body rounded and subcylindrical. Posterior sucker small and terminal in position........... .... *Helobdella elongata* (Castel, 1900)

Body dorsoventrally flattened. Posterior sucker ventral in position......................... .... *Helobdella triserialis* (Blanchard, 1849)

37. Gonopores separated by 2 annuli...................... *Theromyzon maculosum* (Rathke, 1862)

Gonopores separated by 3 or 4 annuli...38

38. Gonopores separated by 3 annuli...................... *Theromyzon rude* (Rathke, 1862)

Gonopores separated by 4 annuli.................... *Theromyzon tessualtum* (O. F. Müller, 1774)

39. 1st pair of eyes closer together than succeeding 2 pairs. Body without papillae. Male and female ducts opening into a common gonopore. Adults less than 15 mm long. Little pigmentation and generally amber coloured.......................... *Glossiphonia heteroclita* (Linn. 1761)

All 3 pairs of eyes equidistant. Body with papillae in 6 longitudinal rows. Gonopores separated by 2 annuli. Adults larger than 15 mm. Deeply pigmented.... *Glossiphonia complanata* (Linn., 1758)

40. 3 pairs of eyes (1 labial and 2 buccal pairs). Gonopores separated by 2 annuli............... .... *Erpobdella punctata* (Leidy, 1870)

4 pairs of eyes (2 labial and 2 buccal). Gonopores separated by 2½ annuli..................... .... *Erpobdella octoculata* (Linn., 1758)

41. Less than 3 annuli between gonopores...42

3 or more annuli between gonopores...43

42. (*a*) 2 annuli between gonopores. Male gonopore surrounded by circle of papillae. Eyes absent. Longitudinal stripes down the body. Ejaculatory duct with long preatrial loop........... .... *Dina anoculata* Moore, 1898

(*b*) 2 annuli between gonopores. No papillae around male gonopore. Either 3 or 4 pairs of eyes. Ejaculatory duct without long preatrial loop............... *Mooreobdella fervida* (Verrill, 1871)

(*c*) Either 2 or 2½ annuli between gonopores. No papillae around male gonopore. 3 pairs of eyes. Ejaculatory duct without long preatrial loop........... *Mooreobdella bucera* (Moore, 1947)*

43. (*a*) 3–3½ annuli between gonopores. Nearly pigmentless or with few dark spots. 4 pairs of eyes. Maximum length 2 cm. Ejaculatory duct with long preatrial loop. Fig. 3...*Dina parva* Moore, 1912
(*b*) 3½ annuli between gonopores. Body heavily blotched with a median dorsal stripe. 4 pairs of eyes. Anus large, opening on conical tubercule. Ejaculatory duct with long preatrial loop..*Dina dubia* Moore and Meyer, 1951
(*c*) 3 annuli between gonopores. 3 pairs of eyes. Ejaculatory duct without long preatrial loop..*Mooreobdella microstoma* (Moore, 1901)

44. Somite VII with *a*3 completely subdivided to give 4 annuli in the somite and with annulus *a*1 of VIII completely subdivided to give 5 annuli. (VII *a*3 is the 13th annulus from the anterior.) Few or no dark blotches on the dorsum, which has a median dark stripe and an orange stripe down each margin. Jaws with 20–25 teeth...................*Percymoorensis lateralis* (Say, 1824)
Somite VII with 3 annuli and somite VIII with 4. VII *a*3 and VIII *a*1 enlarged and sometimes faintly subdivided 45

45. Jaws with 12–16 teeth. Posterior sucker about ½ maximum width of body. Fig. 12A..*Percymoorensis marmorata* (Say, 1824)
Jaws with 10–14 but commonly 11–12 teeth. Posterior sucker about ¾ maximum width of body..................................*Percymoorensis lateromaculata* (Mathers, 1963)*

Acknowledgments

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Meristic Differences between Spring- and Autumn-Spawning Atlantic Herring (*Clupea harengus harengus*) from Southwestern Newfoundland

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Numbers of vertebrae, gill rakers, and of pectoral, anal, and dorsal fin rays of spring- and autumn-spawning Atlantic herring (*Clupea harengus harengus* Linnaeus) in 10 samples from the coastal waters of southwestern Newfoundland were compared. There was no significant difference ($P > 0.05$) between mean vertebral numbers of spring and autumn spawners. Mean numbers of gill rakers and of pectoral, anal, and dorsal fin rays were all higher ($P < 0.01$) for autumn spawners than for spring spawners with gill-raker and pectoral fin-ray numbers exhibiting the greatest degree of difference. It is suggested that the differences in fin-ray numbers between spring and autumn spawners are related to water temperatures during larval development and to differences in developmental rates of spring- and autumn-hatched larvae.

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European investigators have generally used two meristic characters, the number of vertebrae and the number of keeled scales, to characterize different biological groups of herring. Several Canadian investigators have studied the vertebral counts of herring in the western North Atlantic and found no significant differences between mean vertebral numbers of spring and autumn spawners (Jean 1956; Tibbo 1957; Day 1957). However, very little attention has been paid to the number of fin rays in herring racial studies. Schnakenbeck (1927) found that mean numbers of pectoral fin rays were useful for separating herring stocks in the eastern North Atlantic. Dutt (1958) compared the pectoral fin-ray numbers of spring- and autumn-spawning herring in Kiel Bay in the western Baltic and found that autumn spawners had significantly higher pectoral ray averages than spring spawners. Anthony and Boyar (1968) compared the pectoral, dorsal, and anal fin-ray averages of adult Atlantic herring from the Gulf of Maine and adjacent waters but did not investigate possible differences in fin-ray numbers between different spawning groups. Apart from their study, fin-ray numbers have been virtually ignored in studies of herring stocks in the western North Atlantic.

Krefft (1954, 1958) reported differences in the mean numbers of gill rakers of late winter- and autumn-spawning herring in the eastern North Atlantic; late winter spawners had higher gill-raker averages than autumn spawners. Since his study, other German workers have investigated gill-raker numbers in eastern North Atlantic herring. However, variation in gill-raker numbers has not been previously studied in investigations of western North Atlantic herring stocks.

In this paper we present the results of vertebral, fin-ray, and gill-raker counts of Atlantic herring (*Clupea harengus harengus* Linnaeus) taken along southwestern Newfoundland during the 1969 autumn fishery and examine the relation of vertebral, fin-ray, and gill-raker numbers to spawning season. The herring concentrations that overwinter along southwestern Newfoundland from late November to April consist of two spawning groups, autumn spawners making up two-thirds of the population, spring spawners the remainder. Comparison of certain biological characteristics of herring from southwestern Newfoundland and the Magdalen Islands area (Hodder and Parsons MS 1970, 1971) and tagging results (Hodder and Winters MS 1970; Winters MS 1970) indicate that the winter fishery along southwestern Newfoundland is largely dependent on herring that are not indigenous to that area but rather are derived from spring and autumn spawnings in the southern Gulf of St. Lawrence.

Materials and Methods

Ten samples of herring, each containing 50 specimens, were randomly chosen for detailed examination from samples for age and growth studies collected routinely from the catches of purse seiners between La Poile Bay

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and Cape La Hune during late November–early December 1969 (Table 1).

The sex and stage of maturity were determined by gross examination of gonads using the various stages of gonadal development adopted by ICES in 1962 and by ICNAF in 1964 (ICNAF 1964). From 500 specimens collected only four were classed as immature (stage II). Each of the remainder was assigned to one of two categories on the basis of gonadal development. Those with well-developed gonads (stage IV) were designated as spring spawners, and those showing little if any gonad development after a recent spawning (stage VIII) were classed as autumn spawners. Although stage VIII passes into stage III during the gonad developmental cycle, no borderline cases were observed and the distinction between spring and autumn spawners was clear-cut. The immature specimens have not been included in the data analyses.

The gill rakers on the lower branch (hypobranchial and ceratobranchial) of the first left gill arch and the anal and left pectoral fin rays were counted with the use of a binocular microscope. The dorsal fin-ray and vertebral counts were determined from radiographs. All pectoral fin rays and all rakers on the lower limb of the first left gill arch, including rudimentary rakers and the raker in the bend of the arch, were counted. In the dorsal and anal fins all rays including rudimentary rays were counted; the last split rays originating from the same base were counted as one. Vertebral counts excluded the hypural plate.

Results

Of the 496 adult specimens 357 were classed as autumn spawners and 139 as spring spawners. The samples were considered to have been drawn from the same population and thus were combined (Table 2), since analyses of variance showed no significant meristic variation between samples for either spring or autumn spawners. Differences between means for spring and autumn spawners were then tested using the t-test at $P = 0.05$.

There was no significant difference between the mean vertebral numbers of spring and autumn spawners. The mean pectoral and anal fin-ray and gill-raker numbers were higher for autumn spawners than for spring spawners in all samples. The mean dorsal fin-ray numbers were higher for autumn spawners than for spring spawners in all samples but one. The mean pectoral, anal, dorsal, and gill-raker numbers of the pooled data were all higher ($P < 0.01$) for autumn spawners than for spring spawners with the gill-raker and pectoral fin-ray numbers exhibiting the greatest degree of difference.

To test the relation between meristic number and fish size, arithmetic regressions of meristic number on total length were calculated for each meristic character for both spawning groups. Both positive and negative regressions were obtained, and, except in one instance, the regression coefficients did not differ significantly from zero. The regression coefficient of dorsal fin-ray number on total length for autumn spawners (0.007) differed from zero at $P = 0.05$ but not at $P = 0.01$. The corresponding regression coefficient for spring spawners did not differ significantly from the null hypothesis. Since there was no consistent dorsal fin-ray–total length relation, length was not considered to be a source of variation.

TABLE 1. Date and locality of capture, maturity condition, and average length of herring samples taken along southwestern Newfoundland in the autumn of 1969.

Sample no.	Date of capture	Locality	Maturity condition				Avg total length (*cm*)	Length range (*cm*)
			Imm.[a]	Spring spawners	Autumn spawners	Total		
1	Nov. 22	Burgeo	···	10	40	50	33.3	31–36
2	25	Burgeo	1	11	38	50	33.0	27–35
3	25	White Bear Bay	···	13	37	50	32.7	29–36
4	29	Cape La Hune	···	9	41	50	33.4	31–37
5	30	Bay de Vieux	1	14	35	50	32.7	28–35
6	Dec. 1	White Bear Bay	···	14	36	50	33.5	31–36
7	1	Burgeo	···	25	25	50	32.5	29–34
8	4	La Poile Bay	1	10	39	50	32.4	29–37
9	5	White Bear Bay	1	22	27	50	32.8	28–36
10	12	Burgeo	···	11	39	50	33.2	30–36
Totals and avg			4	139	357	500	32.9	27–37

[a]Immature.

TABLE 2. Frequency ranges, mean meristic numbers, and regression coefficients of meristic number against fish length for five meristic characters of spring- and autumn-spawning Atlantic herring from southwestern Newfoundland (samples pooled).

Meristic character	No. fish	Range of frequency	Mean	SE of mean	Regression coefficient	SD of reg. coeff.
Spring spawners						
Vertebrae	137	52–57	55.64	0.06	0.009	0.006
Gill rakers	136	45–51	47.61	0.12	0.021	0.012
Pectoral fin rays	137	16–19	17.18	0.05	0.001	0.005
Dorsal fin rays	127	18–22	19.70	0.06	–0.004	0.006
Anal fin rays	134	16–20	17.58	0.07	0.001	0.007
Autumn spawners						
Vertebrae	359	54–57	55.61	0.03	–0.002	0.002
Gill rakers	349	44–55	49.17	0.09	0.010	0.007
Pectoral fin rays	359	16–21	18.58	0.05	0.003	0.003
Dorsal fin rays	340	18–22	19.95	0.04	0.007[a]	0.003
Anal fin rays	351	15–21	18.17	0.05	–0.005	0.004

[a] Significant at 5% level.

Although the autumn spawners were slightly larger (33.3 cm mean total length) than the spring spawners (32.3 cm), differences in fin-ray numbers were not due to size differences between the two spawning groups.

Discussion

Meristic characters exhibit plasticity under the influence of environmental factors, especially temperature, during the incubation period and early larval life (Schmidt 1921; Vladykov 1934; Tåning 1944; Lindsey 1954; Barlow 1961). An inverse relation between the average number of vertebrae and water temperature for herring has been shown (Hubbs 1925; Rounsefell and Dahlgren 1932; Tester 1937, 1938; Runnström 1941; Bückmann 1950). However, numerous studies have shown a genetic as well as a phenotypic basis for meristic characters in various fish species (Barlow 1961). Although Hempel and Blaxter (1961) demonstrated that the myotome count of Atlantic herring is determined before hatching and that vertebral number is modified by incubation temperature, they also found a considerable difference between vertebral counts for Clyde and German coastal spawners that strongly suggested a genetical difference between the two groups.

In many fish species the number of gill rakers is related to fish size. Krefft (1954, 1958) has shown that there is a positive correlation between gill rakers and length in juvenile herring of northern Europe; however, it is insignificant in adult herring. In the Pacific herring, *Clupea harengus pallasi*, the number of gill rakers is complete when the fish reach 100 mm in standard length (McHugh 1954). The results of the present study indicate that in adult herring from southwestern Newfoundland the correlation between fish length and gill-raker number is negligible.

The relative importance of genetic and phenotypic factors in determination of gill-raker number in Atlantic herring is not known. Krefft (1958) suggested that gill-raker number is genetically fixed and concluded that late winter- and autumn-spawning herring of the eastern North Atlantic, which exhibit highly significant differences in mean gill-raker numbers, are two systematically well-defined subspecies. McCart and Anderson (1967) demonstrated plasticity in kokanee gill-raker number; kokanee reared in an artificial environment had a significantly lower number of gill rakers than their parents. Variation in number of gill rakers has been attributed to temperature variation by some authors (McHugh 1951; Letaconnoux 1954; Lee 1965). However, Andreu (1969), in a comprehensive study of gill-raker variation in *Sardina pilchardus*, has shown that variation in gill-raker number is related to more complex ecological factors, in particular those connected with adaptative phenomena, especially with the optimum utilization of available food. He concluded that polymorphism in gill-raker number in this species is due to genetic differences accumulated with time through the process of natural selection. It has been shown that gill-raker numbers in freshwater coregonids of Sweden and in the Sardinian shad are, to a large extent, genetically determined (Svardson 1952; Cottiglia 1963).

The time of fixation of fin-ray numbers in Atlantic herring is not known. Tåning (1944) reported that the period of fixation for the anal fin in brown trout, *Salmo trutta*, begins in the egg stage but continues after hatching. Subsequently the dorsal and then the pectoral fins are formed. Bigelow and Schroeder (1953) gave 15–17 mm and Blaxter (1962) gave 13–14 mm as the lengths at which the dorsal fin is formed in Atlantic herring. The anal fin is first evident at about 16 mm (Blaxter 1962) and completed at about 30 mm (Bigelow and Schroeder 1953; Lebour 1921).

Although herring eggs develop at colder temperatures in the spring (May–June) than in the autumn (August–September), spring-hatched larvae develop under more favourable environmental conditions during their first months than autumn-hatched larvae. Figure 1 shows the mean monthly sea surface temperatures in the southern Gulf of St. Lawrence for the period from 1963 to 1967 (Anon. 1963–67). It is evident that spring-hatched larvae develop under conditions of increasing water temperature whereas autumn-hatched larvae develop during a period when water temperatures are declining. Thus temperatures at the time of fin-ray fixation are probably higher for spring-hatched larvae than for autumn-hatched larvae. Hubbs (1926) suggested that the number of serial elements in fish is determined by developmental rate. He hypothesized that conditions that accelerate the developmental rate accelerate and accentuate the differentiating tendencies as well as growth. Retarding conditions, on the other hand, reduce both differentiation and growth. Longer developmental periods that may be induced by low temperatures, or other agents that retard development, usually result in higher counts in meristic elements. Jean (1956) has shown that autumn-hatched larvae grow at a slower rate and have a longer period of development than spring-hatched larvae. Hence, autumn-hatched larvae would be expected to have higher mean fin-ray numbers than spring-hatched larvae.

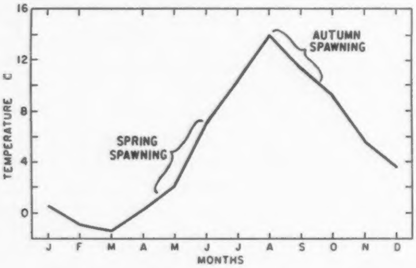

FIG. 1. Mean monthly sea surface temperatures in the southern Gulf of St. Lawrence, 1963–67 (U.S. Navy Oceanographic Office, Washington, D.C.).

Jean (1956) reported that spring- and autumn-spawning herring at Grande-Rivière were identical with respect to vertebral numbers and head length. From these similarities he concluded that each spawning population was a mixture of fish hatched in the spring and fish hatched in the fall. Despite the similarity of mean vertebral numbers of spring and autumn spawners in the present study, we found distinct differences in the mean pectoral, anal, and dorsal fin-ray numbers. If the spring- and autumn-spawning populations were both made up of fish hatched in the spring and of fish hatched in the fall, these morphological characters should be very similar, especially if spring- and autumn-hatched fish were present in about the same proportions among spring spawners and among autumn spawners. The observed differences in fin-ray numbers between spring and autumn spawners correlate with differences in developmental rates of spring- and autumn-hatched larvae. This correlation between the higher fin-ray numbers of autumn spawners and the longer developmental period of autumn-hatched larvae on the one hand, and the lower fin-ray numbers of spring spawners and the shorter developmental period of spring-hatched larvae on the other, supports the conclusion that the majority of autumn spawners are the progeny of herring that spawned in the autumn and spring spawners of herring that spawned in the spring. The differences in fin-ray numbers clearly indicate that spring- and autumn-spawning herring that intermix during the overwintering period along southwestern Newfoundland are not members of a homogeneous group but constitute relatively distinct breeding populations that developed at different times of the year under different environmental conditions

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Seasonal Calorific Values of Freshwater Zooplankton, as Determined with a Phillipson Bomb Calorimeter Modified for Small Samples

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SCHINDLER, D. W., A. S. CLARK, AND J. R. GRAY. 1971. Seasonal calorific values of freshwater zooplankton, as determined with a Phillipson bomb calorimeter modified for small samples. J. Fish. Res. Bd. Canada 28: 559–564.

A modified temperature sensing circuit for the Phillipson microbomb calorimeter allowed accurate calorific determinations on samples as small as 3 cal.

Calorific values of copepods from three lakes in eastern Ontario varied with species, developmental stage, lake, season, and year. No such differences were observed in calorific values for the few species of rotifers and cladocerans tested.

Similar calorific values were obtained for samples preserved by freezing, freeze-drying, and heat-drying, but preservation in formalin resulted in erroneously low calorific values.

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BECAUSE of the difficulty in obtaining monospecific samples of sufficient size for calorimetry, few calorific values for freshwater zooplankton are available in the literature (Comita and Schindler 1963; Richman 1958; Slobodkin and Richman 1961; Sitaramaiah 1967; Wissing and Hasler 1968; Cummins 1967). Many of the determinations were made on animals reared in the laboratory rather than collected from natural environments. Only Wissing and Hasler (1968) have published seasonal calorific values for freshwater plankton, although much of the seasonal variation in their data may be obscured by high variances. Comita and Schindler (1963) and Comita et al. (1966) described seasonal changes in calorific values of the marine copepod *Calanus finmarchicus*.

In this study we developed a modified calorimeter capable of accurately burning small samples, and used the calorimeter to compare summer and winter calorific values of several species and developmental stages of freshwater zooplankton from three small lakes on the Canadian Shield of eastern Ontario.

Materials and Methods

THE CALORIMETER SYSTEM

The standard Gentry-Wiegert modification of the Phillipson (1964) microbomb calorimeter proved insufficiently accurate for the small samples of zooplankton (1–10 mg) available. The time required for preparation of larger samples was prohibitive, and the use of filler material (Richman 1958; Comita and Schindler 1963) is open to several objections, which have been summarized by Phillipson (1964). The following modifications were therefore made to the system.

The thermocouples supplied with the calorimeter were replaced by a circuit of eight Fenwal GB32M2 thermistors (impedance 2K ± 20% at 25 C), connected in series and set in a brass and plexiglass stand (Fig. 1). The thermistors were used as one arm of a conventional resistance bridge, powered by a 1.134-v mercury cell (Fig. 2). The potential difference across the bridge was measured and recorded by means of a Honeywell Electronik 19 recording potentiometer.

OPERATION

The resistance bridge/potentiometer circuit was activated and allowed to warm up for 1 hr before use. The bomb was loaded and pressurized in the normal manner, then cooled approximately to room temperature in a water bath, dried, mounted in the stand inside a thermos flask, and allowed to equilibrate in temperature with the surrounding air. When the rate of potential change approached zero, the bridge was balanced by means of the two variable resistors and the sample was fired. The maximum potential change for each sample was calculated by subtracting the potentiometer reading immediately before firing from the maximum obtained after firing the sample.

CALIBRATION

Pelleted samples of calorimetric grade benzoic acid (6333 cal/g) weighing 0.4–10 mg were combusted, using both the original temperature sensing circuit and the modified design described above. Maximum potential change was plotted against calories released during combustion, and a regression line was calculated in each

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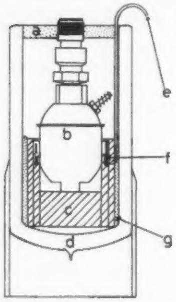

FIG. 1. The modified calorimeter assembled as for firing: *a*, foam rubber insulation; *b*, calorimeter: *c*, plexiglass stand; *d*, vacuum flask; *e*, wires from thermistor ring; *f*, thermistor; *g*, foam rubber packing.

instance. Corrections for prefiring and postfiring changes in potential were applied as outlined by the manufacturer (Gentry-Wiegert Co., Aiken, South Carolina).

SEASONAL SAMPLES

The three lakes sampled were rather different in character:

	Clear	Blackcat	Coon
Area, m^2	88.4	16.0	43.6
Mean depth, *m*	12.5	16.9	2.9
Max depth, *m*	33.0	45.7	18.1
Secchi depth, *m*	9–12	7–10	3–5
Color, *Pt. unit*	0–5	0–5	25–30
*p*H	5.1–7.2	5.0–7.0	6.4–8.0
Conductivity, *μmhos/cm*, 25 C	30	22	130
Lat	45°11′	45°11′	44°36′
Long	78°43′	78°41.5′	78°13′
Comments	Oligotrophic	Meromictic	Slightly dystrophic

Samples were taken with an ordinary plankton net of 70 or 10 μ average mesh diameter. These samples were screened through several bolting silks of different mesh sizes to separate species and larval stages. Examination and hand sorting under a stereomicroscope allowed samples of 98–99% of a given species or stage to be obtained on some occasions. Whenever samples of this purity were obtained, they were preserved, dried at 110 C and kept in a desiccator until calorific analysis was performed. Good separations were obtained frequently in winter, when only a few species and stages were present, but only occasionally in summer due to the much greater diversity of the plankton. Samples that did not allow good separations were discarded. Large species and stages were handpicked with forceps, since a few hundred individuals were enough for an analysis. The fraction of the seston passing through the 70-μ mesh but retained by the 10-μ mesh (referred to here as "microseston") was also dried for calorific analysis.

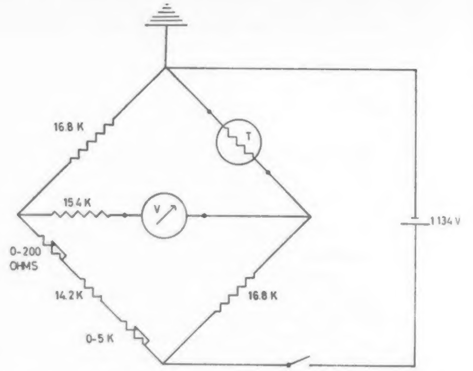

FIG. 2. Resistance bridge circuit for the thermistor unit.

After burning, mean calorific values of the samples were compared by analysis of variance, followed when necessary by Duncan's New Multiple Range Test (Li 1957).

PRESERVATION TESTS

Many workers (personal communications) believe that heat-drying of samples at temperatures high enough to ensure complete dehydration would cause loss of volatile fats, resulting in erroneously low calorific values. We therefore compared the calorific values of freeze-dried and heat-dried samples of copepods in winter and spring, when the animals were fat. Two methods of sample preservation (freezing, formalin preservation) were also tested.

Adult *Diaptomus oregonensis* Lilljeborg were taken from Coon Lake on December 12, 1967. The animals were divided into two samples, one of which was frozen, while the other was preserved in 4% formaldehyde. Calorific determinations on both portions were carried out after 4 months' storage. *Diaptomus oregonensis* adults from April 16, 1968, were dried at 110 C or freeze-dried within 24 hr of capture, and calorific values were determined during the following 48 hr, using the calorimeter described above. All samples were dried in preweighed platinum pans, and stored in a desiccator over calcium chloride after drying. Samples were weighed to 0.002 mg on a Mettler Microgramatic balance.

After combustion in the calorimeter, samples were ashed at 800 C and ash determined by weight. Ash content was less than 2% in all instances tested. Results of the analyses were grouped in pairs according to method of preparation, and the probability of each pair of values being identical was estimated using χ^2 and *t*-tests (Snedecor 1956).

Samples preserved in formalin for 4 months had significantly lower calorific values than frozen ones

TABLE 1. Calorific values (cal/g dry weight without ash corrections) obtained for *Diaptomus oregonensis* using three methods of preservation and heat- and freeze-drying. All values were obtained using the modified calorimeter.

Method of preparation	Date of sampling	Total wt burned (*mg*)	Mean sample size (*mg*)	Mean cal/g	Coefficient of variation %
		Frozen samples			
Heat-dried	Dec. 12	4.210	1.403	$6680 \pm 101^{a,b}$	1.51
Freeze-dried	Dec. 12	3.490	1.745	6750 ± 79	1.16
		Formalin-preserved samples			
Heat-dried	Dec. 12	4.667	1.556	6325 ± 147	2.33
Freeze-dried	Dec. 12	2.691	1.346	6315 ± 54	0.85
		Fresh samples			
Heat-dried	Apr. 16	1.787	0.596	5451 ± 5	0.08
Freeze-dried	Apr. 16	2.126	0.532	5480 ± 278	5.07

[a] One standard deviation of the mean.

[b] Mean calorific values of heat-dried vs. freeze-dried samples were not significantly different for any of the three methods of preservation ($P > 0.95$). Mean calorific values of frozen samples were significantly higher than those of formalin-preserved samples ($P > 0.95$).

(Table 1). There was no significant difference between freeze-dried and heat-dried (110 C) samples regardless of whether drying was performed on fresh animals or those that had been preserved for 4 months.

Results and Discussion

TESTS OF CALORIMETER SYSTEM

Both sensitivity and accuracy were better with the modified calorimeter than with the original unmodified circuit. For samples of benzoic acid of 3–40 cal, our modification gave linear results and low scatter (Fig. 3A). For the modified calorimeter:

$$y = 0.327x + 0.106 \tag{1}$$

with $r^2 = 0.9995$, where x is the calories released during combustion and y is the deflection of the potentiometer in millivolts. The calorific value (C) of an unknown sample in this size range will therefore be

$$C = \frac{y - 0.106}{0.327\ W} \tag{2}$$

where W is the dry weight of the sample in grams, y is as defined above, and C is in calories per gram.

No samples smaller than 18 cal were included in the regression for the original unmodified calorimeter since variances for smaller samples were extremely high. For samples of 18–40 cal

$$y = 0.00634x - 0.0137 \tag{3}$$

with $r^2 = 0.9762$ (Fig. 3B).

SEASONAL VARIATION

All developmental stages of both copepod species increased in calorific value during the winter months, though during the ice-free season relatively low and constant values were found (Table 2). None of the specimens examined were taken from

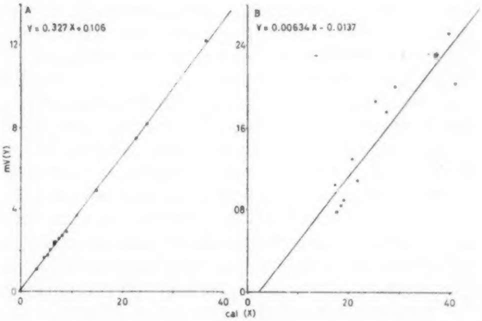

FIG. 3. Maximum potential change (y) in millivolts plotted against calories released by combusted samples (x). Regression lines are according to the equations shown. A, for the modified unit; B, for the original calorimeter.

TABLE 2. Mean calorific values for several developmental stages of two copepod species from three lakes.

Date	Stage	Clear Lake		Blackcat Lake		Coon Lake		Analyses per lake	Mean sample wt, mg
		cal/g dry wt	% ash	cal/g	% ash	cal/g	% ash		
Diaptomus minutus									
Jan. 67	Adult	6985±85[a]	1.1					3	8.040
Feb. 67	Adult	6810±124	1.3					3	6.922
Mar. 67	Adult	6813±86	1.0					3	4.070
Summer 67 (samples from 4 dates, June–Oct.)	Adult	5640±101	2.1	5619±117	0.8	5587±102	0.9	6–12	9.114
	Copepodid	5503±130	1.7	5571±115	0.7	5535±110	0.9	3–7	5.770
	Nauplii	5475±170	0.9	5501±163	1.1	5485±103	1.3	2–5	1.532
Nov. 67	Adult	5580±125	1.3	5593±89	0.8	5629±103	0.9	3–4	8.116
Jan. 68	Adult	6714±65	0.7	6785±107	0.1	6950±131	1.3	3	8.044
Feb. 68	Adult	7008±160	1.2	7375±175	1.4	7001±85	1.9	3	7.068
Mar. 68	Adult	6716±192	1.1	6900±117	2.1	6615±118	2.0	3	3.952
Cyclops bicuspidatus thomasi									
Jan. 67	Adult	6810±124	1.3					3	6.922
Summer 67 (samples from 4 dates, June–Oct.)	Adults & late copepodids	5550±85	0.9	5500±75	1.4	5508±110	1.2	4–7	3.678
Nov. 67	Adults & late copepodids	5465±65	1.5					3	6.132
Jan. 68	Copepodids	6677±79	0.5	6651±134	0.6	6319±194	1.1	3	6.858
Mar. 68	Nauplii	6830±305	1.7	6811	2.1	6855	2.8	1–3	1.014
	Copepodids	7115±129	1.4			7202±104	1.1	3	1.998

[a] One standard deviation of the mean.

lakes that remain free of winter ice cover. Comita and Schindler (1963) and Comita et al. (1966) found that the highest calorific values for the marine copepod *Calanus finmarchicus* from unfrozen seas at similar latitudes occurred in winter months. Summer values recorded here were similar to those published by Comita and Schindler (1963) for other species of copepods.

Calorific values for the two species and various stages of copepods collected from the same lake on the same date were significantly different ($P > 0.95$), as were calorific values for the same species collected from different lakes at the same season ($P > 0.95$) (Table 2). Fatty winter animals were also far heavier (dry weight) than spring or summer animals of the same species. For example *Diaptomus oregonensis* adults from Coon Lake weighed 8.3 μg in December, but only 3.8 μg in April.

The rate of increase in winter calorific value was not the same for the same species in different lakes, or in the same lake in different years. For example, *Diaptomus minutus* adults from Clear Lake gained 7.1 cal/g·day in January–February 1968, whereas adults of the same species from Coon Lake gained only 1.8 cal/g·day and those from Blackcat Lake 17.4 cal/g·day. In January–February 1967, the same species and stage in Clear Lake gained 17.1 cal/g·day, over twice the 1968 rate.

Calorific values of the species of Cladocera and Rotifera investigated did not change from May to December in Coon Lake (Table 3). No seasonal analyses were done for Clear or Blackcat lakes. Both summer and winter calorific values of Cladocera and Rotifera were lower than those of Copepoda for the same dates. There was no significant difference between the calorific values of different cladoceran species for the same dates.[4]

Calorific values of microseston also decreased under winter ice (Fig. 4). Since photosynthesis was undetectably low from January to March (Schindler and Nighswander 1970) this decrease is probably the result of decomposition of algal cells and

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[4] The above statements are based on analysis of variance and a probability ($P > 0.95$) that means for different species, lake, and dates are equal.

TABLE 3. Calorific values for Cladocera and Rotifera taken from two lakes, 1967.

Date	Species	cal/g dry wt	% ash	No. analyses	Avg wt/analysis
		Coon Lake			
May 31	*Holopedium gibberum*	4762±94	7.6	3	7.194
”	*Bosmina longirostris*	4701±104	4.3	3	4.388
”	Mixed *Keratella* and *Kellicottia* sp.	4919±133	5.6	3	1.044
June 21	*Daphnia dubia*	4805±112	8.4	3	5.706
Aug. 2	*Daphnia dubia*	4739±76	7.6	3	5.110
Sept. 13	Mixed *Keratella* and *Kellicottia* sp.	4945±174	7.1	3	1.178
Oct. 9	*Bosmina longirostris*	4812±151	4.8	3	6.886
Nov. 9	Mixed *Keratella* and *Kellicottia* sp.	4785±137	6.5	2	1.832
Nov. 9	*Bosmina longirostris*	4760±133	6.5	3	3.536
Dec. 12	*Bosmina longirostris*	4741±101	4.1	3	3.818
Dec. 12	Mixed *Keratella* and *Kellicottia* sp.	4813±160	8.7	3	2.012
		Clear Lake			
Aug. 21	*Daphnia dubia*	4758±76	9.1	3	5.176
Aug. 21	*Holopedium gibberum*	4718±87	9.7	3	9.864

grazing by zooplankton. Schindler (1968) found that detritus taken from algal cultures grazed heavily by *Daphnia magna* was low in calorific value. The fact that *D. minutus* increased more in calorific value during winter in Clear Lake than in Coon Lake may be related to the higher calorific value of microseston in the former lake. Schindler (1968) found that the efficiency with which detritus was assimilated by daphnids was directly proportional to its calorific value.

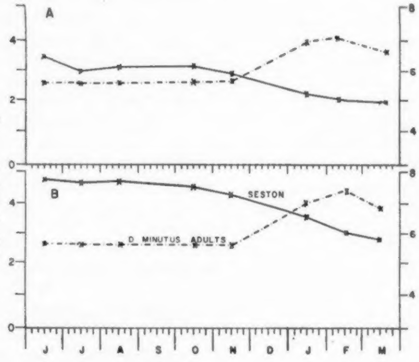

FIG. 4. Seasonal changes in the calorific value of microseston (10–70 μ) and of adult *Diaptomus minutus* 1967–68. A, Coon Lake; B, Clear Lake.

General Discussion

CALORIMETER OPERATION

The modified calorimeter described here allows accurate measurement of the calorific values of extremely small samples. There is, however, some evidence that the relation between potential change and calories released in our modified calorimeter departs from linearity with samples releasing more than 125 cal, though this was not fully tested, and the data obtained are not shown. The thermocouple device supplied with the original calorimeter gave linear results for samples of 100–500 cal, and may be preferable for combusting larger samples. For samples of the small size used here, the thermistor jacket is preferable, since it reaches maximum potential after firing the bomb much more quickly than the original thermocouple; therefore, the percentage error resulting from changes in temperature of the surrounding environment is correspondingly less.

In the results presented here, the external contribution to potential change, as calculated from potential change before firing and after postfiring equilibrium was reached, was less than 1% of the total potential change. This source of error could probably be eliminated or greatly reduced by matching the thermistors in the calorimeter jacket against identical ones in the surrounding air, simulating one desirable characteristic of the original thermocouple circuit while retaining the high sensitivity and speed of the thermistor circuit. Such a modification would be necessary only if most of the samples burned were smaller than 5 cal.

SEASONAL CHANGES IN CALORIFIC VALUE

There has been some speculation about the interpretation of seasonal increases in fat contents of some species. In only one set of circumstances has the adaptive value of increased calorific value been proved, that being the buildup of premigratory fat reserves by birds (Odum et al. 1965). Slobodkin and Richman (1961) hypothesized that calorific differences might be of taxonomic value. Cummins (1967) believed that highest seasonal calorific values occurred during times of egg production. Our data suggest that fat increases in adult freshwater copepods precede, rather than accompany, egg production. A decrease in calorific value accompanies the production of eggs by *D. minutus*. Comita and Schindler (1963) found relatively low calorific values for *Diaptomus arcticus* egg sacs (5672 cal/g). Eggs were not abundant enough in our samples to allow calorific values to be determined.

Populations of *D. minutus* in all three lakes appeared to carry greater numbers of eggs under ice (when animals had higher calorific values) than during the ice-free season (Schindler unpublished data). Judging from numbers of fat droplets in the bodies of the animals, females producing eggs in late winter under the ice decreased in calorific value at that time, whereas those producing eggs after spring breakup appeared to remain fat throughout the winter. This may be why calorific values for March samples of adult *D. minutus* are lower than those for January and February samples. Unfortunately, data for April, during the spring ice breakup, were impossible to obtain due to unsafe ice.

Since the winter increase in fat content of Copepoda takes place in juveniles as well as adults, it cannot be related solely to reproduction. It is more likely that the increase has survival value, allowing the individual to survive or even grow during periods of low food abundance or poor food quality, which are common in midwinter when ice is covered with deep snow, and again after ice-out in spring, when phytoplankton concentrations that appear under snow-free ice in late winter are dispersed by spring overturn.

Judging from our observations on numbers of fat droplets in the bodies of winter specimens of several species of Copepoda from several locations, winter increase in calorific value is widespread in Canada and the northern United States. We have not examined material from south of 44°N lat. Further study of seasonal calorific values at several latitudes is needed for all major groups of freshwater zooplankton.

Acknowledgments

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Electrical Responses of the Olfactory Epithelium of Atlantic Salmon (*Salmo salar*)

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Multiunit spike activity was recorded from the olfactory mucosa of Atlantic salmon (*Salmo salar*) using metal-filled glass microelectrodes. A number of simple and complex solutions were tested for stimulatory effectiveness. Amino acids as a class of compounds were highly stimulatory; little or no response was obtained to a variety of simple sugars, *n*-aliphatic acids, alcohols, or amino-substituted alcohols. An increase in asynchronous spike activity was evoked by low concentrations of amino acids and oscillatory activity was evoked by higher concentrations of amino acids. Brief treatment of the epithelium with dilute solutions of $HgCl_2$ blocked the chemosensitivity of the receptors.

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WITH the exception of the work of Shibuya (1960), who investigated slow potential changes in the olfactory mucosa, little information is available concerning the electrical properties of the olfactory epithelium of teleosts. Therefore, electrophysiological methods were developed to record from the epithelium of young salmon, to compare the chemical sensitivity and specificity of olfactory receptors with similar information that has been obtained from other vertebrates. Of interest also were the possible differences in specificity between olfactory and gustatory receptors in the same species (Sutterlin and Sutterlin 1970).

Materials and Methods

Atlantic salmon (*Salmo salar*) were obtained as smolts from a local hatchery where they had been hatched from native breeding stock taken from the Northwest Miramichi River, N.B. They were held in large tanks under natural photoperiod for approximately 5 months during which time they were maintained on beef liver and commercial fish food. At the time of experimentation they had reached a length of 15–18 cm.

The fish were immobilized with tubocurarine, fixed in a Plexiglas holder, and perfused through the mouth throughout the experiments. The flap of skin covering one naris was removed to expose the olfactory rosette. The rosette was then perfused with water (3.5 cc/min) from a continuous-flow delivery tube. The delivery tube was directed toward the center of the rosette, which remained submerged. Fluid from the delivery tube, after passing over the lamellae, passed out of the olfactory cavity. In some fish the olfactory bulb was also exposed by removing a portion of the cranium and aspirating the underlying fat and connective tissue.

Constant volumes of aqueous dilutions of odorants were drawn into disposable glass capillary tubes and injected into the stream of water perfusing the epithelium (Fig. 1). The water used both for perfusion and dilution of the various odorants tested was the same as that which supplied the holding tanks. Although the exact concentration of odorant at the specific location of the electrode on the epithelium was not known, dye tests and calculated dilutions occurring in the delivery tube and olfactory cavity indicated that the maximum concentration was probably no lower than 1/50 the concentration stated. All serial dilutions, however, are relative to each other by a concentration factor of 10. Possible influences of solvent contaminants, temperature, or tactile disturbances were always eliminated by testing the solvent water alone.

Woods metal-filled glass capillaries were prepared according to Dowben and Rose (1953) and used for recording from the olfactory epithelium. Multiunit discharges were amplified by conventional capacitatively coupled preamplifiers and led into a Grass (model 7P3A) amplitude-frequency integrator (integrating time constant: 0.5 sec). This integrator was in turn coupled to a Grass (model 7P10A) d-c integrator to measure the accumulated activity throughout the discharge. Bipolar silver–silver chloride electrodes were employed for recording oscillatory potentials from the olfactory bulb.

Results

ELECTRICAL RESPONSES FROM THE OLFACTORY EPITHELIUM

The olfactory rosette of salmon smolts consists of a series of radiating primary lamellae from which secondary lamellae project (Fig. 1). Exploration of the lamellar surface with microelectrodes reveals spontaneous spike activity (Fig. 2A) between

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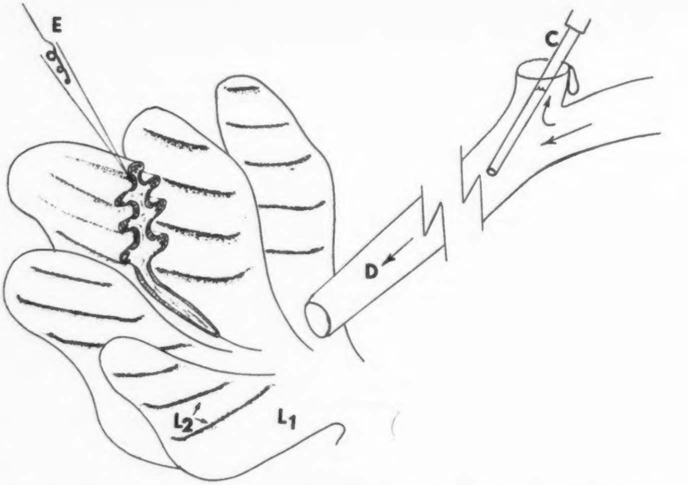

FIG. 1. Diagram of a portion of the olfactory rosette of a salmon smolt showing the electrode (E) positioned between secondary lamellae (L_2). Primary lamellae (L_1), the tip of the delivery tube (D), and capilliary tube (C) containing odorant are also shown.

secondary lamellae and a complete absence of such activity at the tips of the primary and secondary lamellae. These active and inactive regions correlate well with the histological localization of sensory and secretory regions described by Pfeiffer (1963) in Pacific salmon. Histological sections of the olfactory epithelium of Atlantic salmon smolts show similar cellular organization to those of the Pacific salmon mentioned above.

During chemical stimulation with low concentrations of amino acids, a rapid increase in spikelike activity occurs (Fig. 2B). This asynchronous activity increases as the concentration of stimulant is elevated. At high concentrations of the more stimulatory amino acids (Table 1) large amplitude oscillatory potentials (30–40 cycles/sec) replace the asynchronous activity (Fig. 2C). Such oscillatory activity adapts much more rapidly to repetitive stimulation than the asynchronous activity and long interstimulus intervals must be employed to maintain the oscillations. This activity is not dependent on a possible centrifugal influence of the olfactory bulb, nor is it due to electrotonic spread from the olfactory bulb, because mucosal oscillations are two to three times the frequency of bulbar oscillations (Fig. 3A) and, in any event, sectioning the ipsilateral olfactory nerve does not influence the mucosal oscillations (Fig. 3B).

Oscillatory behaviour of the olfactory mucosa has been observed in a number of species: rabbit (Adrian and Ludwig 1938), toad and frog (Takagi and Shibuya 1960), and turkey vulture (Shibuya and Tucker 1967). This activity may be due to spike synchrony among adjacent cells sharing supporting cells as common current sources. Types of junctional complexes, possibly serving as low-resistance pathways, have been described between receptor and supporting cells in fish (Bannister 1965; Gemne and Døving 1969).

Although isolation of single unit responses is difficult to achieve, presumably due to recruitment of nonspontaneously active units, several units were isolated during this study. In all instances, increases in firing frequency were observed (Fig. 5) during stimulation with amino acids and increases in spike amplitude always occurred as firing frequency became elevated. Activity from two units was maintained long enough to test two amino acids, threonine and histidine. Each unit responded to both compounds.

The effects of heavy metals as blocking agents have been demonstrated in a number of chemosensitive systems (Deonier 1938; Yur'eva 1957; Hidaka and Yokota 1967; Sutterlin and Sutterlin 1970). Mercuric chloride and lead nitrate were tested in this study and only the former was effective. The chemical sensitivity of the olfactory receptors to all odorants tested (10 amino acids, human hand rinse, and food extracts) is blocked for at least 1 hr after a brief treatment with 10^{-2}-

10^{-4} M $HgCl_2$ (Fig. 4). However, the degree of background spontaneity of the preparation is not altered. Mercuric ion is known to tie up sulfhydryl groups in proteins and the blocking effect observed here may be due to interference with receptor sites or enzymatic components of the receptor membranes.

The possibility that the olfactory stimulants used in this study somehow alter the conductivity of the medium at the electrode tip, creating a recording artifact, not associated with increased cell firing, can be excluded for several reasons. First, the majority of effective stimulants employed were neutral amino acids at the isoelectric point and it is difficult to see how such dilute solutions could effect the conductivity of the medium in logarithmic fashion with changes in concentration (Fig. 6). Secondly, a change in conductivity at the electrode tip caused by the odorants should enhance the amplitude of the unaltered level of spontaneity after blockage with $HgCl_2$ and this is not the case. Thirdly, an increase in firing frequency of single units during stimulation definitely exists. A possible explanation for the associated change in spike amplitude is that during excitation, axonal or somatic spikes may invade the dendritic portion of the cell to a greater degree and propagate nearer to the electrode.

CONCENTRATION–RESPONSE RELATIONS

The majority of amino acids tested were stimulatory (Table 1). Concentration–response relations were determined for several of these amino acids using different fish. L-Alanine, L-threonine, and L-proline were chosen to provide information from compounds of widely differing stimulatory effectiveness. Multiunit discharges and their integrations show that stimulatory effectiveness

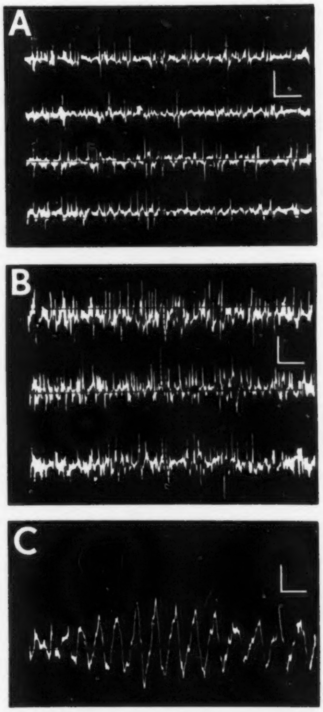

FIG. 2. Types of electrical activity recorded from the olfactory epithelium: A, sample traces of spontaneity from many units during continual perfusion with tap water; B, traces of asynchronous activity from the same preparation evoked by 10^{-6} M DL-serine; C, oscillatory potentials from the same preparation evoked by 10^{-3} M DL-serine. Time, 50 msec. In A and B, amplitude $50 \mu v$; in C, amplitude $200 \mu v$.

TABLE 1. Relative stimulatory effectiveness[a] of some amino acids tested on the olfactory epithelium at 10^{-4} M.

	No. preparations	Peak activity	Total activity
5-Amino valeric acid	8	1.2	1.0
L-Hydroxyproline	13	3.3	2.2
Taurine	12	3.3	2.8
L-Proline	12	5.8	4.2
D-Amino butyric acid	8	6.2	4.2
D-Threonine	9	7.4	4.2
DL-Isoleucine	13	9.7	5.4
D-Alanine	9	10.0	6.0
L-Arginine	6	12.0	6.7
DL-Valine	12	16.2	11.7
DL-Threonine	9	19.0	13.0
DL-Phenylalanine	12	19.6	15.5
L-Threonine	13	20.9	17.0
DL-Leucine	11	24.6	17.4
Glycine	13	27.4	19.1
L-Cystine (10^{-5} M)	4	27.7	19.6
Methionine	6	29.5	22.4
DL-Serine	13	32.3	22.7
L-Histidine	12	33.0	27.5
DL-Alanine	13	33.7	31.3
L-Alanine	10	34.2	32.0

[a] Data analyzed for analysis of variance for single factor experiments with repeated measures (irregular sample size) and the difference between means tested using standardized range statistics (Winer 1962). Compounds that are separated in rank by at least five other compounds evoke significantly different responses (99% confidence level).

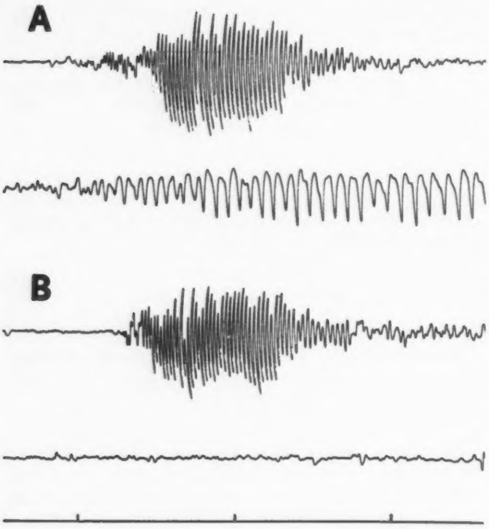

FIG. 3. Simultaneous recordings of oscillatory potentials from olfactory mucosa and bulb evoked by 10^{-3} M DL-serine. In A, mucosal potentials (upper trace) are higher in frequency than bulb potentials (lower trace). In B (same preparation), the olfactory nerve was sectioned, eliminating bulb potentials, but without influencing mucosal oscillations. Time, 1-sec intervals. Recording time constant is the same for both amplifiers.

varies linearly with the log of the concentration of the amino acid (Fig. 6). Preparations exhibiting oscillatory activity were excluded from analysis because of the discontinuous nature of the activity treated by the integrating methods. Regression lines were fitted by least squares method and the concentration intercept determined for six fish tested with the three compounds. The mean values obtained for L-alanine, L-threonine, and L-proline are 3.2×10^{-9} M, 2.5×10^{-6} M, and 3.2×10^{-5} M, respectively. The log slope ratios among the three different compounds when measured in the same fish do not significantly differ from unity. Whether or not such treatment is of value in determining absolute or relative thresholds as related to olfactory acuity remains to be determined when other methods have been employed as a basis of comparison.

RELATIVE STIMULATORY EFFECTIVENESS OF DIFFERENT AMINO ACIDS

Table 1 ranks, in order of increasing stimulatory effectiveness, a number of amino acids tested at the same concentration. A variety of complex stimuli were also found to be effective: stagnant water, aqueous extracts of commercial fish pellets and liver, water from tanks in which other salmon were held, human saliva, and human hand rinses. Few such responses were quantified, but very high sensitivity was observed, for example, to human hand rinse. A 2-sec finger dip in 1 liter of water

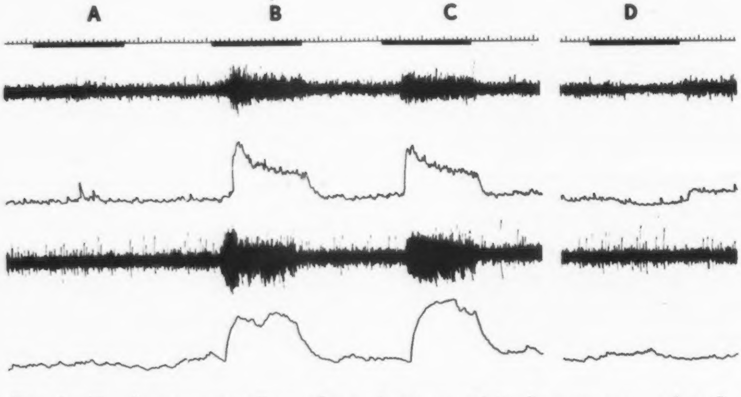

FIG. 4. Simultaneous recordings of asynchronous activity from mucosa and oscillatory potentials from bulb showing blockage of receptor sensitivity with Hg^{++}. Upper trace indicates time intervals of 1 sec and periods of stimulation; traces 2 and 3, mucosal activity and its integration; traces 4 and 5, bulb activity and its integration. A, treatment with water from glass beaker containing 100 ml tap water; B and C, treatment with same water after 5-sec finger dip; D, treatment with same finger-rinse water after 10-sec treatment with 10^{-3} M $HgCl_2$ and 30 sec of rinsing.

was sufficient to evoke a detectable response. Table 2 lists a group of compounds that were found nonstimulatory. Some of these compounds are effective stimulants for terrestrial vertebrates; many are amino-substituted compounds that were tested in light of the pronounced stimulatory effectiveness of amino acids.

ADAPTATION STUDIES

When subjecting the mucosa to either closely spaced pulses or a continual application of odorant, adaptation was apparent. Differential rates of adaptation for individual odorants were not carefully examined, but interstimulus intervals greater than 2 min were sufficient to exclude complications due to adaptation. Attempts to selectively adapt the mucosa to certain amino acids and also to several complex stimuli always resulted in some degree of cross-adaptation (Fig. 7).

BULBAR RESPONSES

Olfactory bulb responses were examined simultaneously with mucosal responses in 12 fish. Oscillatory responses from the bulb were quite variable from time to time in the same fish and among different fish. In general, however, solutions judged as stimulatory from mucosal responses resulted in oscillatory bulbar responses.

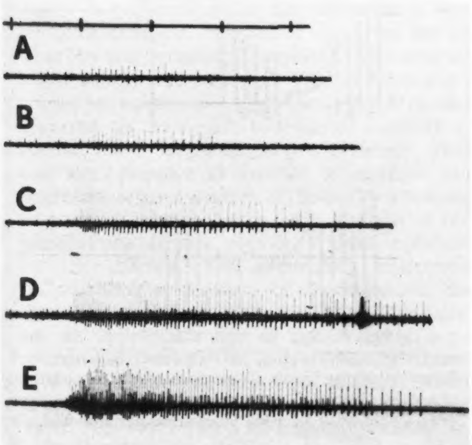

FIG. 5. Response of a single unit to increasing concentrations of L-histidine: A, 10^{-7} M; B, 10^{-6} M; C, 10^{-5} M; D, 10^{-4} M; E, 10^{-3} M. Time intervals on upper trace 1 sec.

Discussion

ANATOMICAL CONSIDERATIONS

The fine structural detail of the salmon olfactory epithelium has not been investigated, and the possibility of more than one morphological type of receptor cell cannot be overlooked. Receptor cells having terminal cilia, terminal microvilli, and terminal rods have been described in other species of fish (Bannister 1965). Gemne and Døving (1969), however, describe the receptor cells in the burbot as being of a single type. Innervation of the olfactory epithelium in the Atlantic salmon is reported to include only the olfactory nerve. The trigeminal and terminal nerves do not seem to enter the epithelium (Gawrilenko 1910). It is known

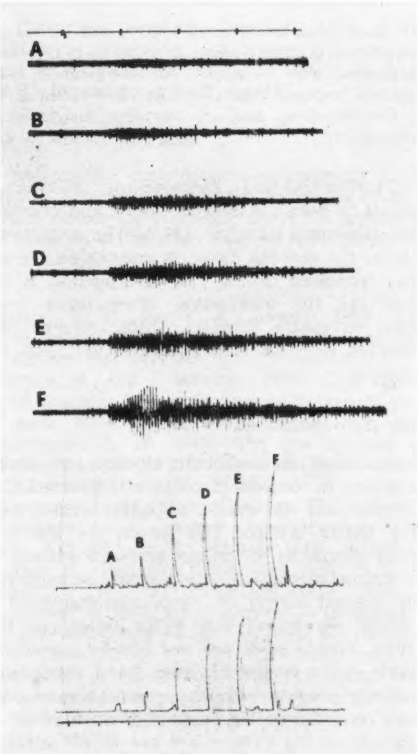

FIG. 6. Multiunit response patterns to increasing concentrations of L-alanine. A, 10^{-8} M; B, 10^{-7} M; C, 10^{-6} M; D, 10^{-5} M; E, 10^{-4} M; F, 10^{-3} M (notice beginnings of oscillatory activity in F). Time trace indicates 1-sec intervals. The lower pair of traces shows the integrated peak activity and accumulated activity (A, B, C, D, E, F) of the above responses. Time intervals 30 sec.

TABLE 2. Nonstimulatory[a] compounds tested on the olfactory epithelium.

Propionic acid
Valeric acid
Caproic acid

Sucrose
Lactose
Dextrose

Methanol
Ethanol
Propanol
Butanol
Pentanol

Morpholine

Dimethylamine
Trimethylamine
n-Butylamine
sec-Butylamine
1,4-Diaminobutane

Ethanolamine
L-2-Amino-1-propanol
3-Amino-1-propanol
L-Dimethylamino-2-propanol
3-Dimethylamino-1-propanol
L-2-Amino-butanol
D-2-Amino-1-butanol
5-Amino-1-pentanol

[a] All compounds tested at 10^{-3}, 10^{-4}, and 10^{-5} M and responses compared with reference solutions of L-proline, DL-threonine, and L-alanine. All compounds listed evoked less response than L-proline when tested at the same concentrations and are therefore designated as nonstimulatory.

that in turtles and birds, chemosensory information is relayed by both the olfactory nerve and branches of the trigeminal (Tucker 1963). The anatomical nature of the nervous elements responsible for the activity measured during this investigation is not certain, but the localization of excitable areas between secondary lamellae where receptor cells are located provides some evidence that they are the source.

AMINO ACID SPECIFICITY

Normal short chain aliphatic alcohols with amino substitution in various places are not stimulatory and neither are the smaller aliphatic alkanes containing amino groups. The reason for the pronounced sensitivity to amino acids in relation to other compounds tested may possibly be explained by the limited variety of compounds employed in this study. Because of their polar nature and low volatility, amino acids are not usually considered odorants in the terrestrial sense. Land vertebrates, by necessity, employ the gustatory sense for detection of such compounds. The taste buds on the roof of the mouth of the salmon are not at all sensitive to amino acids but are sensitive to the "odorous" homologous series (C_1–C_6) of *n*-aliphatic acids (Sutterlin and Sutterlin 1970). However, this lack of sensitivity to amino acids is not the case for gustatory activity in other fishes (Bardach et al. 1967). There is some behavioural evidence that salmon in fact do smell other types of compounds: eugenol at 0.18 ppm (Tarrant 1966), and morpholine at 10^{-6} ppm (Hasler 1954). Morpholine was tested during this investigation and found to be nonstimulatory at concentrations of 0.9 ppm.

Behavioural evidence indicates that the amino acid L-serine is smelled by salmon and that it is one of the active components of the skin rinse of mammalian predators that have a repellant effect on migrating salmon (Idler et al. 1956; Brett and MacKinnon 1954). It is noteworthy that DL-serine was not especially stimulatory in relation to several other amino acids (Table 1), yet the discriminatory capabilities of salmon between serine and several other amino acids have been demonstrated (Idler et al. 1956). It remains to be determined whether or not free amino acids themselves or free amino

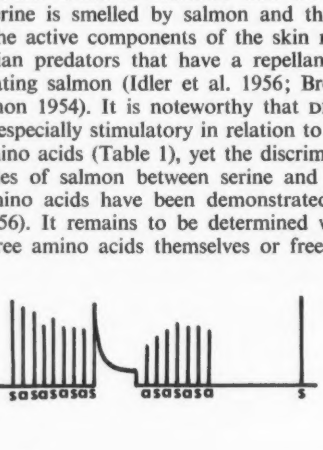

FIG. 7. Cross-adaptation of mucosal activity with different odorants. Each of the above four preparations was pulsed alternately with odorant every 15 sec until a near stable level of peak integrated activity was observed. A period of continual stimulation by one of the compounds was followed by alternate testing of the pair. *a* = DL-alanine, 10^{-4} M; *s* = DL-serine, 10^{-4} M; *h* = L-histidine, 10^{-4} M; *g* = glycine, 10^{-4} M; *r* = human hand rinse. Tracings have been drawn to compress record.

groups, perhaps in soluble proteins or other molecules, have any other special significance in olfactorily mediated salmon behaviour.

The sensitivity of primary olfactory neurons has been examined in a number of vertebrates (snakes, tortoise, birds, and mammals) (Moulton and Tucker 1964). They all show about the same relative sensitivity to a group of test odorants. The restriction of the fish to an entirely aquatic existence, however, imposes some limitations on the chemical spectrum that can be detected. Døving (1966) found that nonvolatile compounds were somewhat more effective than volatile compounds in altering the level of spontaneity in single bulbar neurons in the burbot.

Altner and Boeckh (1967) describe for the frog receptors a basic spectrum of chemicals to which all cells respond. An additional small but variable side spectrum was also observed for each cell. Perhaps amino acids as a class of compounds compose such a basic spectrum for the salmon nose, thus accounting for the large integrated responses to amino acids in relation to those compounds designated as nonstimulatory.

Differences in Stimulatory Effectiveness of Amino Acids

For the majority of amino acids used, the manufacturers claimed only 98–99% purity. Although attempts were made to obtain the purest commercially available compounds, it is entirely possible that even the smallest amount of impurity could distort or even account for the differences in stimulatory effectiveness observed. If this is not the case, what physical or chemical properties of the molecules might explain the ranking? Without becoming involved in multifactor correlations, very little seems apparent. Of the D- and L- forms of threonine and alanine, the L- forms are more stimulatory. Molecular size, presence or absence of aromatic characteristics, relative position of the amino substitution or presence, absence, or relative position of other substitutions do not provide a clear explanation for the differences in stimulatory effectiveness.

Concerning mechanisms of discrimination, there is reason to believe that the above question is not an appropriate one to ask. Varying degrees of chemical specificity of single olfactory receptors have been shown in a number of studies (Gesteland et al. 1963; O'Connell and Mozell 1969; Shibuya and Tucker 1967). This is most probably the situation in the salmon nose, but whether or not different receptors or groups of receptors have differential sensitivity to individual amino acids as a single class of compounds cannot be resolved at this time. The cross-adaptation studies performed in this investigation do not differentiate between possible overlapping in chemical specificity among different receptors and similar receptors with less specificity. Single unit studies were accomplished with great difficulty and the data are not sufficient to shed any light on this problem.

The varying degree of employment of the olfactory sense by different species of fish (e.g., in food selection, predator–prey interactions, species recognition, migration, and territorial recognition), the diversity in anatomical arrangements of olfactory structures, and the variations in salinity in which the chemoreceptive membranes must function provide some fascinating problems in fish olfaction.

Acknowledgments

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Note Added In Proof

Independent, contemporaneous studies by N. Suzuki and D. Tucker on the olfactory epithelium of the freshwater catfish are amazingly similar in approach and conclusions to the study reported here (personal communication).

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A Technique for Enumerating Kokanee Salmon (*Oncorhynchus nerka*) Fry Migrating through Streams, with an Appendix for Processing Catch Data by IBM 360 Fortran IV Computer Programs

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ACARA, A. H., AND H. D. SMITH. 1971. A technique for enumerating kokanee salmon (*Oncorhynchus nerka*) fry migrating through streams, with an appendix for processing catch data by IBM 360 Fortran IV computer programs. J. Fish. Res. Bd. Canada 28: 573–585.

A technique for enumerating kokanee salmon (*Oncorhynchus nerka*) fry migrating downstream was developed in Meadow Creek (a tributary to the Duncan River and Kootenay Lake, B.C.) and was used to obtain an estimate of 10.06 million kokanee fry in 1968. The hyperbolic relation between the nightly mean catch per net per minute (C) and nightly probable error (E) as the percent of the nightly total was expressed by $\log E = -0.5703 - 0.4568 \log C$; E was largest at the beginning and end, and smallest at the peak of fry migration. E was also calculated as the percent of the seasonal total, and in 1968 was ±5.8%. Of this total, ±0.7% was accumulated during April, when 6.4% of the migration occurred; ±3.9% was accumulated in May, when 82.2% of the migration occurred; and the final ±1.2% was accumulated in June, when 11.2% of the migration occurred. The variability in abundance of fry and spacing of groups of fry are considered to be the most important factors controlling the magnitude of error.

Some aspects of migratory behaviour of kokanee fry in Meadow Creek were revealed and their effects on estimates of abundance are discussed.

A series of IBM 360 Fortran IV computer programs for processing catch data are also given in an appendix.

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MEASURES of salmonid production in streams can be made in several ways, such as counting adults, redd sampling, and by fry and smolt enumerations. The numerical relation between spawners and their returning progeny, over a series of brood years, is of considerable value in managing the stocks. From these data, recruitment curves can be drawn from which estimates of the optimum number of fish in spawning escapements can be taken and consequently provide a rational basis for establishing the number of fish to be harvested (Ricker 1958). Since a good deal of the variability in such relations is attributable to losses during the early stages of development — between egg deposition and migration of fry in particular — a measure of the fry produced from a known egg deposition can be used for forecasting purposes.

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Indices to fry abundance without associated statistical error have often been obtained by fishing with small nets in the migration paths between the spawning beds and the lake or estuary for which the fry were bound (Vernon 1966; Todd 1966; Hartman et al. 1967; Foerster 1968). For best results, nets should be operated from fixed structures such as bridges or weirs spanning the stream so that regular fishing schedules can be maintained regardless of stream conditions. Furthermore, such a facility should ordinarily facilitate an estimate of the statistical error associated with the estimates.

In 1965, the spawning grounds of kokanee (*Oncorhynchus nerka*, landlocked sockeye salmon) in the Duncan River, B.C., were rendered inaccessible by construction of the Duncan Dam by British Columbia Hydro and Power Authority (Fig. 1). To compensate for this loss, a spawning channel was constructed adjacent to Meadow

Creek in 1967, and production studies were undertaken of the Duncan–Lardeau stocks as a whole.

This paper describes the method used to estimate the numbers of fry migrating from the Meadow Creek system, nightly and seasonally, and provides an estimate of the probable error associated with these fry enumerations in 1968.

In addition, some aspects of the migratory behaviour of kokanee fry were recorded and are summarized in this paper.

A series of computer programs has also been developed to process catch data obtained by this method (Appendix 1).

Sampling Site, Device, and Procedures

The site was the "hatchery fence" immediately below the lower limits of spawning. Therefore, estimates of fry abundance include fry produced in the spawning channel as well as in natural spawning areas above and below the channel (see Fig. 1).

Kokanee fry (mean length 2.5 cm) were captured in a series of three sampling devices each consisting of an elongated rigid metal frame located with its long axis perpendicular to the stream and supporting a column of nets each with openings 1.0 × 0.5 ft. All were fitted with nylon marquisette with a mesh size 3/8″ to form a bag 2.0 ft deep. Catches made in these nets formed the basis of the daily and seasonal estimates. Nets were fished hourly from dusk to sunrise throughout the vertical water column and individual catches were obtained and recorded in each 1-ft increment from the stream bottom to the surface at three locations about 10 ft apart and equidistant from the banks, and each other across the fence (Fig. 2). Fishing stations 1, 2, and 3 were located in the center of right, midstream, and left sections, respectively, of the stream. Fishing was done at least every other night and for part of every hour throughout the diel pattern of the run. The fishing interval varied with the abundance of the fry. It was 10 min every hour at the beginning and end of the migration and reduced to 5, 2, and 1 min as the abundance increased. Estimates of the number of migrants passing hourly in each foot of water in the three sections of the river were calculated from the catch data. These estimates showed changes in horizontal and vertical distribution of the fish.

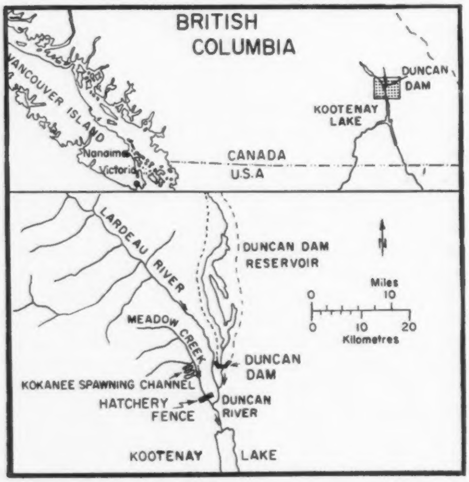

FIG. 1. Duncan–Lardeau River system, showing location of Meadow Creek and kokanee fry sampling site.

The totals of fry passing downstream on sampling nights were estimated from total catch data at all three stations in accordance with the fractional part of the stream cross section and the fraction of the migration period that was fished.

The seasonal total was obtained by summing the estimates obtained on nights when fishing was conducted with those calculated for nights when no fishing was conducted. The latter were obtained by interpolating between the totals of the two closest nights of fishing.

Water velocities were measured at middepth at each fishing station by means of a Gurley pygmy current meter, and discharge components were calculated by multiplying the velocity readings by the areas of the river sections. During the season, water velocities varied between 3 and 5 ft/sec. No artificial light was used during fishing periods.

CALCULATION OF FRY ABUNDANCE

Hourly abundance (C_H) in each 1-ft level at each location was calculated from the equation

$$C_H = \frac{S}{S_N} \times \frac{60}{T_F} \times C,$$

where S is the cross-section area in square feet (see Fig. 2), S_N is the area of the net opening in square feet, T_F is fishing time in minutes, and C is the catch.[1]

Taking $S_N = 0.5$ ft² and $S = 10$ ft², the equation becomes

$$C_H = \frac{1200}{T_F} \times C \text{ or } C_H = K \cdot C,$$

where K is a constant and its value is 120, 240, 600, and 1200 for 10, 5, 2, and 1 min fishing each hour respectively. K must be recalculated, or rechecked for each sampling location on a stream.[2]

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[1] Because water levels vary, the portion of the top net that is submerged also varies. However, the expression $\frac{S}{S_N}$ is a constant at any water level and no adjustment is needed as the river discharge changes.

[2] In Meadow Creek spawning channel, $S = 12$ ft² and K was 144, 288, 720, and 1440.

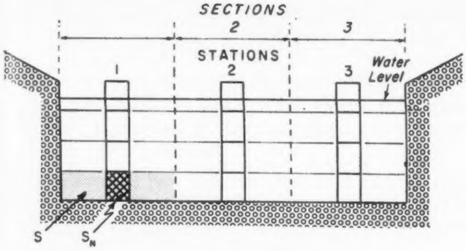

FIG. 2. Location of three sampling devices in stream profile.

Sources of Error

There could be two sources of error in the estimates of the number of fry migrating in a sampling night: (1) catch efficiency of the nets and (2) variation in migration intensity within hourly periods caused by variations in temporal and spatial distribution of the fry in the river profile. Both were examined in some detail in 1966 and 1968.

CATCH EFFICIENCY OF THE NETS

The catch efficiency of a net may be considered to be 100% if it catches and holds all the fry it intercepts. That is, none must detect and avoid the net and none must swim back out of it. The catch efficiency of the standard net of 0.5 ft² opening and 2-ft deep bag was tested against that of nets of 1.0, 2.0, 3.0, and 4.0 ft² opening, and the same 2-ft deep bag size, by fishing the standard net side by side with a larger net and expressing their efficiencies (%) by:

$$E = \frac{C_T}{C_S} \times \frac{A_S}{A_T}$$

where C_T and C_S are catches in the test and standard nets, and A_S and A_T are the areas of the standard and test nets respectively.

The efficiencies were calculated for each test (Table 1). The mean catch efficiencies were 0.75 for 1 ft², 0.69 for 2 ft², 0.75 for 3 ft², and 0.73 for 4 ft² test nets. The lower efficiencies of the larger nets may be caused by the surface areas of the bags used on the larger nets not being sufficiently large to pass all the water entering them; in that event, some fry would be held out by back pressure from the net, and others might be carried back out of the net as a result of turbulence. Nets of larger surface area would pass sufficient water and no loss in efficiency should then result.

The catch efficiency of the standard net was also tested against a stationary trap with tapering sides and an opening of the same size by fishing it side by side and touching during 1-min periods.[3] During 20 tests, catches varied between 12 and 49 fry for the standard net, and 14 and 48 for the stationary trap per 10-min fishing period, according to variation in the abundance of fry in the river, but catches in the two nets never differed by more than two fry in any 10-min period, and the mean catches were identical (28 fry). This supports the contention that the efficiency of the standard net was 100%.

The bags were the same length on all nets and could conceivably have had an equalizing effect upon the efficiencies of the nets. To test these, three standard nets each with openings 0.5 ft² but bag sizes 18, 24, and 36 inches were fished side by side and touching for 10 periods of 10 min each (Table 2). Catches in all three nets (bag sizes 18, 24, and 36 inches) were within the 99% confidence limits of the mean catch, which showed they had the same efficiency when fished with the identical net opening (0.5 ft²).

VARIABILITY OF CATCH IN EACH HOUR

As mentioned previously, hourly estimates were made from the catch obtained at the beginning of each hour. Variability in catches within hours was measured as follows.

The standard net was fished for 10, 5, 2, or 1 min in midstream, starting at 7:00 PM (PST) and repeated at 15-min intervals until 5:00 AM in two series — once during the first half, and once during the second half of the run (after the seasonal peak of downstream migration). The fishing periods and catches are shown in Table 3. Catches were never identical in four successive fishing periods within an hour, but when all catches, beginning on the even hours or at any common time after the hour, were summed over an entire night (totals near right margin of Table 3), they invariably fell within the 99% confidence interval of the mean total catch. Nightly standard deviations varied from one to five fry. The greatest probable error was ±11.7% and occurred during the 10-min fishing periods of April 22–23, when fry abundance was lowest. The error varied from ±2.4% to ±6.9% of the nightly totals thereafter.

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[3] The stationary trap was 8 ft long, constructed from galvanized screen panels of 3/8″ mesh on a metal frame that tapered slightly from 1.0 × 0.5 ft at the front to a smaller opening into a live box at the back. Water velocities were a constant 3.5 ft/sec both inside and adjacent to the trap during the tests. Thus fry were unable to swim back out of the trap and are believed not to have avoided it.

Because the greatest range in catches usually occurred at midstream where the nets were fished, the error can be considered maximum.

Relation between Nightly Probable Error and Fry Abundance

Total nightly fry catches per minute and probable associated errors (see Table 3) were plotted in Fig. 3. This relation can be expressed as

Table 1. Fry catches in standard (0.5 ft² opening) and test nets, and calculated efficiencies of four test nets in 1967. The data are shown as standard net catch over test net catch (e.g., 16/18). To calculate efficiency, this expression is multiplied by the factor expressing the increase in area of opening of the test nets over the standard net (i.e., 2, 4, 6, or 8).

	Net opening (*square foot*)							
	1.0		2.0		3.0		4.0	
Date	Catch	Efficiency	Catch	Efficiency	Catch	Efficiency	Catch	Efficiency
Apr. 24–25	16/18	0.56	22/ 62	0.70	35/147	0.70	36/173	0.60
	15/20	0.67	12/ 38	0.79	29/181	1.04	29/180	0.78
	21/38	0.90	17/ 41	0.60	19/ 80	0.70	31/151	0.61
Apr. 26–27	22/33	0.75	14/ 34	0.61	31/150	0.81	34/211	0.78
	26/56	1.08	13/ 36	0.69	25/ 95	0.63	36/180	0.62
	31/42	0.68	12/ 35	0.73	29/225	1.29	19/101	0.66
	33/49	0.74	36/101	0.70	30/108	0.60	21/121	0.72
Apr. 28–29	31/44	0.71	42/130	0.77	24/103	0.72	38/238	0.78
	16/24	0.75	44/123	0.70	41/175	0.71	45/272	0.76
	13/20	0.77	46/129	0.70	46/185	0.67	41/287	0.88
	12/16	0.67	26/ 62	0.60	48/291	1.01	43/285	0.83
	10/16	0.80	24/ 77	0.80	52/256	0.82	57/342	0.75
May 2–3	36/58	0.81	23/ 76	0.79	38/134	0.59	31/211	0.85
	42/64	0.76	21/ 61	0.73	25/105	0.70	30/181	0.75
	41/65	0.79	15/ 37	0.62	30/105	0.58	21/105	0.63
	37/60	0.81	16/ 43	0.67	19/ 71	0.62	23/120	0.65
May 4–5	28/34	0.61	17/ 44	0.65	15/ 75	0.83	25/150	0.75
	24/34	0.71	13/ 33	0.63	19/ 82	0.72	26/159	0.76
	27/39	0.72	18/ 44	0.61	21/ 80	0.63	24/151	0.79
	33/44	0.67	19/ 60	0.79	15/ 65	0.72	19/107	0.70
Mean		0.75		0.69		0.75		0.73
Range		0.61–1.08		0.60–0.80		0.58–1.29		0.60–0.88
SD		0.1		0.2		0.1		0.1

Table 2. Variability in fry catches in standard nets (0.5 ft² opening) fitted with three bag sizes during 10, 10-min fishing periods, June 14, 1968.

			Catch			
18″ bag	24″ bag	36″ bag	Mean catch	SD	SE	99% confidence interval
6	7	5	6	1	1	3–9
10	11	12	10	1	1	7–13
15	14	13	13	1	1	10–16
8	9	7	8	1	1	5–11
20	21	20	20	1	1	17–23
19	18	17	17	1	1	14–20
15	12	19	15	3	2	10–20
13	12	11	12	1	1	9–15
11	11	12	11	1	1	8–14
8	9	7	8	1	1	5–11

in 1968.

Date	Time fishing started (*min*)	Hours of samplings (PST) 7:00 PM	8:00 PM	9:00 PM	10:00 PM	11:00 PM	12:00 PM	1:00 AM	2:00 AM	3:00 AM	4:00 AM	5:00 AM	Total
Series 1													
10-min fishing periods													
Apr. 22–23	00	0	7	13	13	7	3	3	6	1	1	0	54
	15	0	7	16	16	8	4	5	7	1	0	0	64
	30	1	8	14	17	9	2	3	5	0	0	0	55
	45	0	9	17	13	7	4	4	6	1	0	0	65
										Mean catch			60
										SD			5
										SE			3
										99% confidence interval			53–67
										Probable error			7
										Probable error as % of mean			11.7
5-min fishing periods													
May 10–11	00	0	0	0	33	35	18	6	8	14	5	1	120
	15	0	0	0	32	39	14	8	6	13	5	0	115
	30	0	0	0	35	32	17	6	7	11	4	0	110
	45	0	0	2	29	39	13	7	5	12	3	1	106
										Mean catch			113
										SD			5
										SE			3
										99% confidence interval			106–120
										Probable error			7
										Probable error as % of mean			6.2
2-min fishing periods													
May 13–14	00	0	0	1	17	21	17	11	8	8	10	1	93
	15	0	0	0	19	19	16	8	7	8	8	2	87
	30	1	2	0	18	21	15	12	7	7	8	0	92
	45	0	0	1	17	20	16	11	6	6	9	0	85
										Mean catch			89
										SD			3
										SE			2
										99% confidence interval			85–93
										Probable error			4
										Probable error as % of mean			4.5
1-min fishing periods													
May 19–20	00	0	0	11	37	48	26	13	15	16	7	1	174
	15	0	0	10	35	47	25	14	16	14	6	0	167
	30	0	1	9	33	51	24	12	15	15	8	0	167
	45	0	1	9	36	49	25	14	17	17	6	0	172
										Mean catch			170
										SD			3
										SE			2
										99% confidence interval			166–174
										Probable error			4
										Probable error as % of mean			2.4

TABLE 3. Fry catches in each hour, during 10-, 5-, 2-, 1-min fishing periods. The sampling started 7:00 PM and was repeated at 15-min intervals until 5:00 AM in two series: (1) during the first half (between April 22 and May 20) and (2) during the second half (between May 23 and June 16) of the seasonal migration in 1968. — (*Concluded*)

Date	Time fishing started (*min*)	Hours of samplings (PST)											Total
		7:00 PM	8:00 PM	9:00 PM	10:00 PM	11:00 PM	12:00 PM	1:00 AM	2:00 AM	3:00 AM	4:00 AM	5:00 AM	
						Series 2							
						1-min fishing periods							
May 21–22	00	0	1	22	26	23	18	10	8	7	5	0	120
	15	0	1	21	25	21	17	11	7	7	6	1	116
	30	0	0	20	24	21	16	12	6	8	6	0	113
	45	0	1	19	22	22	17	10	8	6	7	1	113
										Mean catch			116
										SD			3
										SE			2
										99% confidence interval			112–120
										Probable error			4
										Probable error as % of mean			3.4
						2-min fishing periods							
May 23–24	00	0	0	0	8	11	9	7	8	5	3	1	52
	15	0	1	0	10	12	8	8	7	6	4	0	56
	30	0	0	2	9	13	7	7	7	4	2	1	52
	45	1	0	1	8	11	8	7	6	5	3	0	50
										Mean catch			53
										SD			2
										SE			1
										99% confidence interval			50–56
										Probable error			3
										Probable error as % of mean			5.7
						5-min fishing periods							
May 29–30	00	0	0	15	20	12	13	8	7	11	8	0	94
	15	0	0	14	27	15	15	9	6	12	6	0	104
	30	0	0	18	19	16	16	7	8	12	5	0	100
	45	0	0	18	28	14	17	8	6	13	5	0	107
										Mean catch			101
										SD			5
										SE			3
										99% confidence interval			94–108
										Probable error			7
										Probable error as % of mean			6.9
						10-min fishing periods							
June 15–16	00	0	4	6	14	8	2	2	1	4	0	0	41
	15	0	3	5	15	9	2	3	1	2	0	0	40
	30	0	2	4	16	9	3	3	1	3	0	0	41
	45	0	4	7	14	8	4	4	0	4	0	0	45
										Mean catch			42
										SD			2
										SE			1
										99% confidence interval			39–45
										Probable error			3

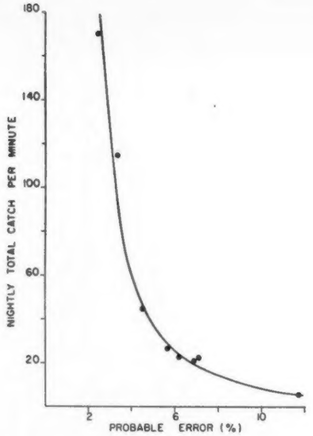

FIG. 3. Nightly total catch per minute and probable error as percentages of nightly totals.

$$\log E = -0.5703 - 0.4568 \log C \; (r = -0.99),$$

where E is nightly probable error (%) and C is nightly mean catch per net per minute of fishing time. This equation can be used to calculate the nightly probable error as the percent of fry abundance when the mean catch per net per minute is known. In addition, the probable seasonal error can be obtained by applying the nightly probable errors to the estimates of the percent of total fry passing downstream each night and accumulating them over the entire season. Figure 3 can also be used when the mean catch per net per minute is known, to regulate nightly fishing time to hold probable error below some required level.

Enumeration of Kokanee Fry in 1968

TIMING AND ABUNDANCE

In Meadow Creek, the spawning migration begins during the second week of August, spawning usually occurs in September and October, and fry emerge from the spawning gravel and migrate to Kootenay Lake the following spring.

Kokanee fry were sampled as described above at the "hatchery fence" on Meadow Creek in 1968 (Fig. 1). The migration started early in April, reached its midpoint between May 10 and 20, and was essentially complete by June 24. It totalled 10.1 million fry (Fig. 4).

Nightly errors, expressed as the percent of nightly totals, were calculated from the mean nightly catch per net per minute and the equation given in the previous section, and plotted in Fig. 5A. Also plotted were estimates of nightly fry output expressed as a percent of the seasonal total (10.1 million). Finally, to obtain the nightly error as the percent of seasonal total as shown in Fig. 5B, the nightly error as the percent of nightly total was multiplied by nightly estimates as the percent of seasonal total (see Fig. 5A). The cumulative probable error for the Meadow Creek fry enumeration in 1968 is also shown in Fig. 5B. This was ±5.8% of the total downstream migration. Of this, ±3.9% was accumulated during May. Only ±0.7% was accumulated in April, and the remaining ±1.2% was accumulated in June. In contrast, the nightly errors as percentages of the nightly totals were lowest during May and highest at the beginning and end of the migration (Fig. 5).

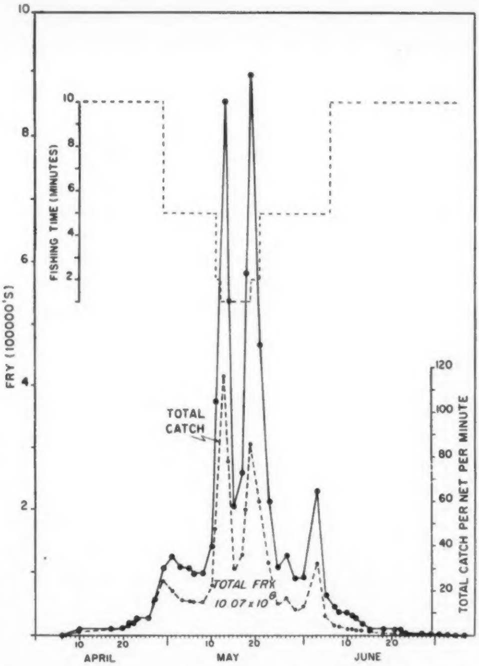

FIG. 4. Numbers of kokanee fry migrating nightly from Meadow Creek, as estimated from samples taken at hatchery fence, fishing times, and mean catch per net per minute in 1968.

The cumulative seasonal migration indicated that 6.4% of the total fry migration occurred between April 6 and 30, 82.2% between May 1 and 31, and 11.4% between June 1 and 30 (Fig. 6). Two abrupt peaks of abundance were measured: the first on May 13 accounted for 9.0% and the second on May 19 accounted for 8.5% of the total run (Fig. 5).

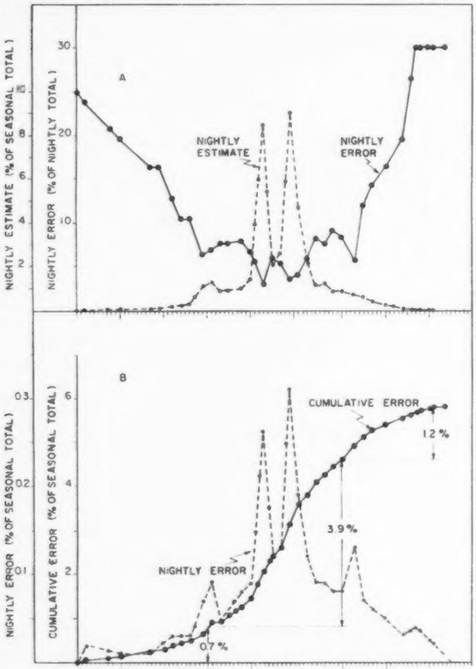

FIG. 5. Nightly estimated fry as percentages of the seasonal total, nightly errors as percentages of nightly totals (A) and nightly and cumulative errors as percentage of the seasonal total (B).

The timing of this run may vary somewhat from one year to the next, so the sampling should preferably be conducted daily during the peak of migration.

MIGRATORY BEHAVIOUR

Migratory behaviour of Pacific salmon juveniles is apparently influenced by river discharge, temperature, light intensity, precipitation, and a number of other less easily measured features of the environment, such as silt load and river profile (Hoar 1951, 1954; McDonald 1960; Coburn and McCart 1967).

The behaviour of Meadow Creek fry changed during the 1968 migration as shown by the horizontal, vertical, and diel distributions.

Horizontal and vertical distribution

The average number of fry migrating through each of the three sections of the creek (Fig. 2) and in 1-ft increments from the bottom were grouped in nine 10-night periods and expressed as a percentage of the Meadow Creek total in each period in Table 4. The discharge within each section, depth of water, and daily mean water temperature increased, and are also shown.

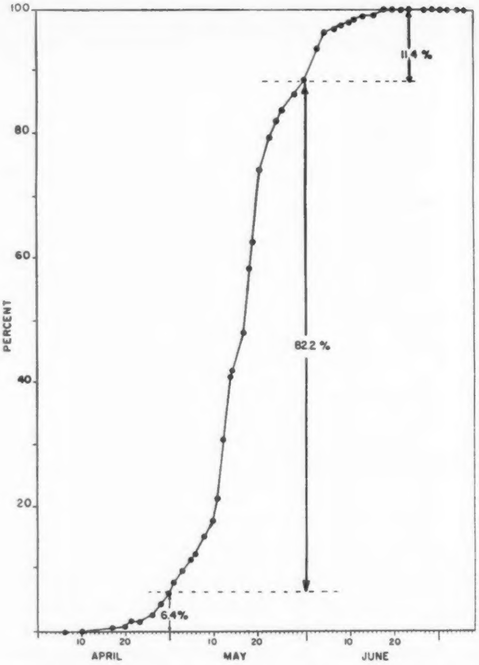

FIG. 6. Cumulative seasonal migration of kokanee fry in percent through Meadow Creek in 1968.

Horizontal distribution of fry across the creek channel varied through the season (Table 4). During the season, a mean of 43.1% of the total passed downstream in the midsection, where the greatest percentage (36.6%) of the total discharge occurred. The only marked exception to this pattern of greatest fry movement associated with greatest discharge occurred during the April 1–10 period, when both fry abundance and discharge were low. Then, the greatest fry migration occurred in section 1, where the discharge was lowest. Increased discharge in mid-May was associated with a marked increase in fry abundance generally.

The vertical distribution of fry was not always the same in the three sections (Table 3). The abundance was greatest at middepth. During May 1–20, fry abundance was greatest at the bottom; it increased at middepth during May 21–June 29 when fry abundance was between 52.3 and 40.2% of

ABLE 4. Fry distribution as a percent of the total migrating in three horizontal and four vertical divisions of the stream profile, scharge through the three horizontal divisions, and mean water levels and temperatures in 10-day periods at the hatchery fence, eadow Creek, 1968.

		April			May			June				
		1–10	11–20	21–30	1–10	11–20	21–30	31–9	10–19	20–29	Mean	%
		Horizontal fry distribution (%)										
ct. 1		42.3	23.7	17.2	20.3	17.3	23.1	17.5	14.9	16.5	21.4	
ct. 2		32.7	40.2	41.4	47.6	46.8	43.3	38.6	52.3	44.9	43.1	
ct. 3		25.0	36.1	41.4	32.1	35.9	33.6	43.9	32.8	38.6	35.5	
otal		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
		Vertical fry distribution (%)										
-ft levels												
h ft		0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	5.1		
d ft		0.0	0.0	0.0	0.0	0.0	4.1	19.4	18.2	25.3		
d ft		0.0	0.0	0.0	14.5	44.2	52.3	44.4	49.5	40.2		
t ft (bottom)		100.0	100.0	100.0	85.5	55.8	43.6	34.2	32.3	29.4		
otal		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0		
		Mean and range in discharge (ft³/sec)										
ct. 1	Mean	17	17	19	49	78	103	148	125	164	80	31.1
	Range	15–19	15–19	17–25	43–61	67–85	84–182	92–180	112–140	131–197		
ct. 2	Mean	24	24	32	50	100	127	167	140	183	94	36.6
	Range	22–26	22–26	24–52	40–50	92–110	108–146	141–195	117–163	125–241		
ct. 3	Mean	23	23	22	39	80	114	153	127	164	83	32.3
	Range	21–25	21–25	20–30	26–54	65–99	88–136	86–210	99–163	113–215		
otal	Mean	64	64	73	138	258	344	468	392	511	257	100.0
	Range	60–64	62–66	70–98	114–168	224–292	277–397	303–581	328–466	369–653		
		Mean and range in water level (ft)										
	Mean	1.0	1.0	1.1	1.7	2.3	2.7	3.4	2.9	3.7		
	Range	0.9–1.1	0.9–1.1	1.0–1.4	1.5–1.7	2.1–2.7	2.4–3.0	2.3–3.9	2.6–3.4	3.2–4.0		
		Mean and range in water temperature (C)										
	Mean	6.5	4.7	7.6	7.6	7.9	8.5	8.6	8.5	8.6		
	Range	6.4–6.6	4.6–4.8	6.8–8.2	6.2–8.8	7.7–8.3	7.5–8.9	8.2–9.2	8.5–9.1	8.5–8.7		

the total in the second foot level, when the water level increased to 3 and 4 ft. This indicates that the vertical distribution of fry was determined in large part by the volume of water transported at intermediate depths in midstream in Meadow Creek.

Diel migration

Hourly fry abundance during 24-hr fishing periods demonstrates the predominantly nocturnal pattern of fry movement, and mean hourly catches are expressed as percentages of the nightly totals in four separate nights in Fig. 7. The nocturnal patterns are shown as means of successive periods of 10 nights of fishing at the three stations in Fig. 8. All migration was confined to the hours between 7:00 PM and 7:00 AM and two daily peaks of abundance usually occurred; the first peak between 10:00 and 12:00 PM, the second between 2:00 and 3:00 AM. The first peak was always larger than the second. In May and June nightly migrations were shorter than in April and the first peak had by then shifted to 10:00–11:00 PM. In April, between 64% and 82% of the nightly totals occurred between 9:00 PM and 1:00 AM, and in May and June these had increased to between 85 and 100% during the same 4-hr interval (Fig. 8). This phenomenon is due to the increase in daylight during the seasonal migration in spring, which shortens the period of fry movement. It has been observed commonly in the studies of migrating salmonids.

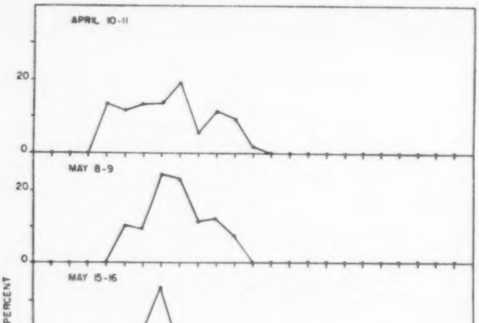

FIG. 7. Diel periodicity of downstream movement of kokanee fry in Meadow Creek in 1968.

Comparison of diel migrations

The nightly migration pattern may be shown by the percent of the total catch occurring in each of the 13 hr of fishing. The pattern can then be compared with the average pattern during the 10-day period that includes it. The departure of nightly migration patterns from the mean pattern of 10-day periods were tested by chi-square (using mean hourly percentages during the 10-day periods as observed values and percentages from individual nights as estimated values). The results are plotted in Fig. 9. The results indicate that, in all but four tests, there was a probability of 0.85 or better that the disparity was due to chance. One exception occurred at the beginning and one at the end of the season, and the other two on May 15 and 17. On May 13 estimated nightly abundance was 857,640 fry; this decreased to 209,400 fry on May 15, and 258,120 fry on May 17 (see Fig. 4 and 8). The diel patterns on May 15 and 17 were similar to the mean pattern obtained for the May 21–30 period rather than the May 11–20 period; when they were tested against the pattern in that period, they gave values of 7.84 and 6.94 for May 15 and 17 respectively, and were again within the 85% probability level. The large disparity between expected and observed patterns of migration on May 15 and 17 may be accounted for by the fact that the downstream migration was relatively small and brief on those nights and was similiar to the situation during the decline in abundance in the May 21–30 period.

Discussion

The studies outlined above revealed two important sources of error in the estimates of nightly and seasonal migration of kokanee fry. These were the catch efficiency of the nets, and variation in migration intensity. The latter was considered to be primarily due to behavioral patterns of the fish.

CATCH EFFICIENCY

Differences in the catch efficiency of standard nets and nets of larger than standard size appeared to be explained by the lower ratio of area of net opening to bag surface area in the latter. The ratio of these measurements is shown in Fig. 10 for nets with a constant ratio (line A) and for the series of nets used in this study (line B). Errors due to net efficiency can probably be overcome by maintaining the relation shown in Fig. 10, line A, or by applying empirically derived corrections.

VARIATION IN MIGRATION INTENSITY

The probable error of the nightly fry estimates was highest at the beginning and end of the run and lowest near the midpoint of the run, when abundance was highest. The size and spacing of groups of fry, therefore, appeared to be the most important source of error during the run. There are a number of factors governing the size and spacing of groups. For instance, it is common knowledge that spawning salmonids show strong preferences for parts of the stream having particular stream bed gravel sizes, water velocities, and water temperatures. These and the flow of water through the gravel influence the survival rates of eggs and fry and govern the spacing of the fry initially. Later salmonid alevins move laterally and vertically within the gravel (Bams 1969) and this movement is more pronounced in coarse gravel than in fine (Dill and Northcote 1970). Some further modification of the patterns of abundance doubtless occurs as a result of this. Salmonid fry are photopositive (Hoar 1951, 1954; Hoar et al. 1957), but their eyes become semidark-adapted, as the evening light decreases more rapidly than their eyes can respond. This causes them to move upward through the gravel and then lose visual contact with their surroundings, and they are displaced downstream.

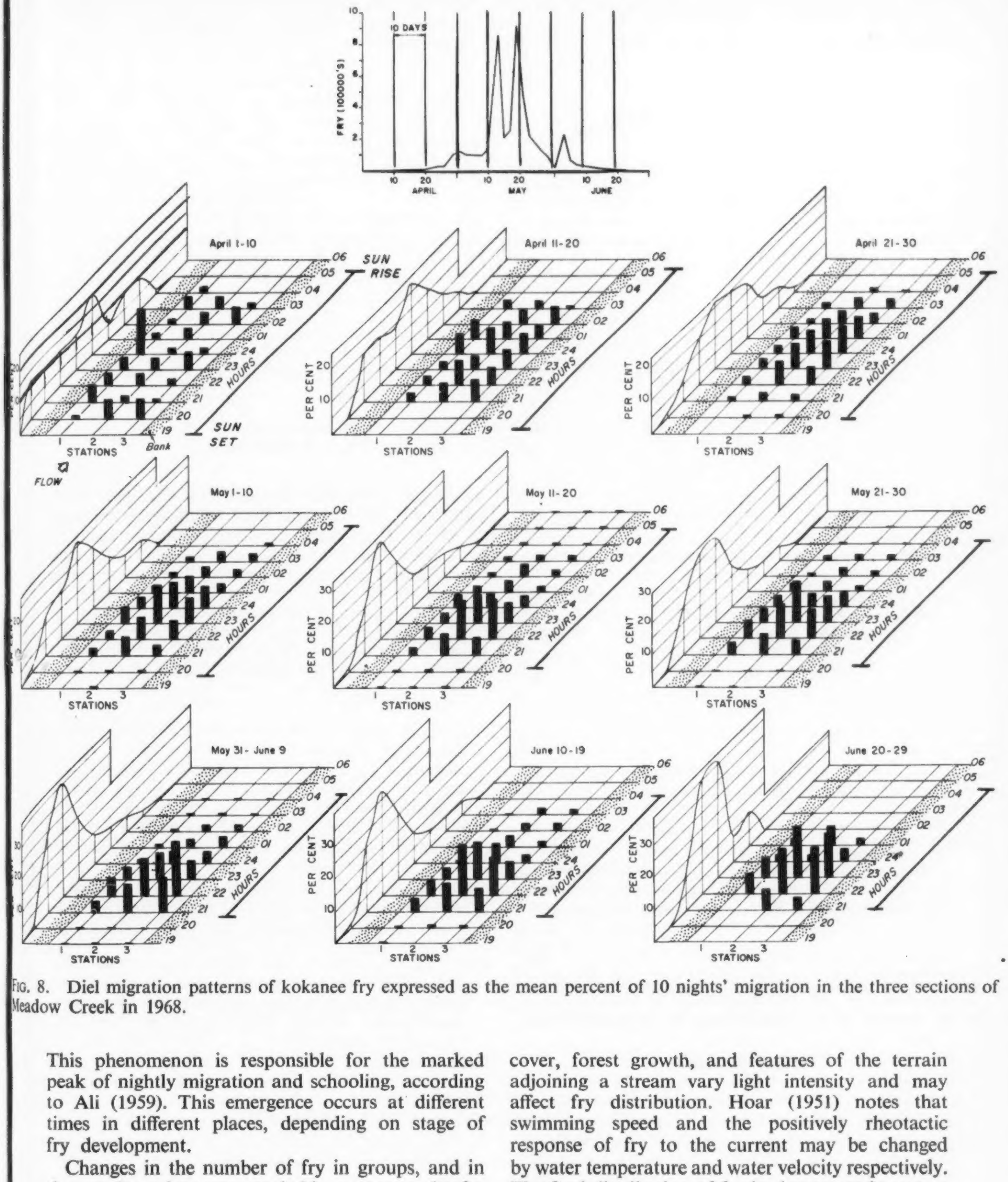

FIG. 8. Diel migration patterns of kokanee fry expressed as the mean percent of 10 nights' migration in the three sections of Meadow Creek in 1968.

This phenomenon is responsible for the marked peak of nightly migration and schooling, according to Ali (1959). This emergence occurs at different times in different places, depending on stage of fry development.

Changes in the number of fry in groups, and in the spacing of groups, probably occurs as the fry move downstream; for instance, variable cloud cover, forest growth, and features of the terrain adjoining a stream vary light intensity and may affect fry distribution. Hoar (1951) notes that swimming speed and the positively rheotactic response of fry to the current may be changed by water temperature and water velocity respectively. The final distribution of fry in the stream is seen as the end product of a series of adjustments to their

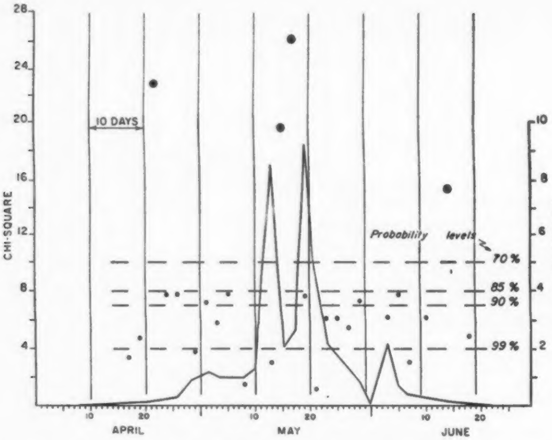

FIG. 9. Results of comparisons of nightly and 10-night mean patterns of kokanee fry migration in Meadow Creek 1968.

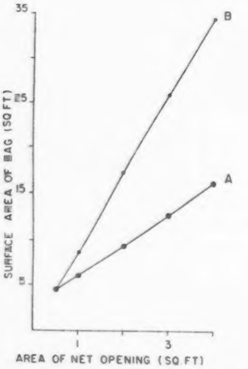

FIG. 10. Relations between the area of net openings and the surface area of bags used in tests (A) and the surface area of bags required to maintain a constant ratio of net opening to bag surface area (B).

complex environment. This distribution is controlled by the size and spacing of the groups of fry, and this sets the magnitude of probable error in estimating fry abundance in Meadow Creek.

Acknowledgments

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Appendix 1

A Series of IBM 360 Fortran IV Computer Programs for Fry Enumeration in Streams

A. H. ACARA[1] AND A. TWEEDALE[2]

A series of computer programs was developed to process catch data obtained by the method described above. The programs produce printed and plotted nightly and seasonal totals, and horizontal, vertical, and nocturnal distribution of fry, and its variability in 10-day periods.

CAPACITY OF PROGRAMS

The programs are designed to handle measurements with the following limitations: (1) maximum number of sampling hours per night is 13; (2) maximum number of water levels is 8; (3) maximum number of sampling stations is 5.

However, number of levels and hours can be increased by changing the dimensions of the arrays used in the programs.

INPUT REQUIREMENTS

The programs require, as punched-card input data, the quantities that appear in the equation shown in the section describing calculation of fry abundance. The identifications are the location and date. Further identification may be added.

PROGRAMS

Program 1 — Reading and printed listing of input cards and transfer card images to magnetic tape disc.

Program 2 — Basic detail output processing of nightly results in tables as hourly number of fry in each location for each level, as the total of levels in each location in each hour, and as the percent of nightly total and cross section in each hour.

Program 3 — Conversion of all sampling dates into a numeric form suitable for extrapolation and interpolation for no sampling nights.

Program 4 — Extrapolation and interpolation of nightly results to estimate nightly production, cumulative totals, nightly abundance as the percent of seasonal total, nightly errors as percentages of nightly totals, and the seasonal total and cumulative error for the season. Plotting of production, cumulative total, and cumulative percentage against sampling dates.

Program 5 — Dividing into 10-night periods of the total sampling period, calculating 10-night average diel patterns as the percent of nightly total and cross section. Taking expected value as the 10-night average, calculating deviations and chi-square values for sampling dates in each 10-night period.

Program 6 — Plotting of nightly diel patterns.

Program 7 — Plotting of 10-night average diel patterns.

Program 8 — Plotting of daily temperature (0 C).

Program 9 — Calculating daily discharges (ft^3/sec) in each cross section, plotting discharges for each station and cross section daily.

Programs 1, 2, and 3 would normally be run in sequence. Programs 4 through 9 can then be used as required.

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[2] Computing Center, University of Victoria, Victoria, B.C.

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Factors Influencing Scope for Activity and Active and Standard Metabolism of Rainbow Trout (*Salmo gairdneri*)[1]

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Utah Cooperative Fishery Unit
Utah State University, Logan, Utah, USA

DICKSON, I. W., AND R. H. KRAMER. 1971. Factors influencing scope for activity and active and standard metabolism of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Bd. Canada 28: 587–596.

Scope for activity was similar for hatchery and wild rainbow trout (*Salmo gairdneri*), except at 25 C where values for wild trout were significantly higher than those for hatchery trout. In hatchery and wild rainbow trout, respectively, scope for activity was highest at 15 and 20 C. Active metabolism increased with temperature to 570 and 592 mg O_2/kg per hr at 15 and 20 C, respectively. At 25 C, active metabolic rates of wild trout were higher than those for hatchery trout. In hatchery and wild trout, respectively, standard metabolism increased from 36 and 42 mg O_2/kg per hr at 5 C to 138 and 120 mg O_2/kg per hr at 25 C.

Scope for activity of hatchery trout was highest after 6 days starvation. Starvation had no effect on active metabolism of hatchery trout, but decreased their standard metabolism after 2 days.

Scope for activity and active and standard metabolism of wild trout were similar during forenoon to those during afternoon when light simulated natural day and night and also when lighting was constant for 24 hr.

Active metabolism of hatchery trout was higher during the spawning period than other periods of the year and was consistently the higher for males throughout the year.

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THIS study was undertaken to (1) determine and compare scope for activity of catchable-size hatchery and wild rainbow trout throughout their temperature tolerance range and (2) at the temperature for which scope for activity was highest, to describe the effects of starvation, time of day, sex, and season on rainbow trout metabolism. Numerous factors may influence standard or active metabolic rates, or both, and can therefore affect scope for activity (Fry 1957). These terms have recently been defined by Fry (1967). Scope for activity has been considered as a means to assess environmental stress on fishes (Brett 1958), as an index of the energy available for swimming fish (Brett 1964), and, more recently, as an index to total energy expenditure by fish (Beamish and Dickie 1967). It was used in our study primarily to compare the effect of temperature on the respiratory metabolism of hatchery and wild trout and to show the effect of other factors on rainbow trout metabolism.

Little has been done to describe scope for activity and standard and active metabolic rates throughout the temperature tolerance range for a particular species of fish, a notable exception being the study of sockeye salmon (*Oncorhynchus nerka*) by Brett (1964). Even less attention has been given to respiratory studies of particular fish strains though differences in growth and survival of domestic and wild trout reported by Flick and Webster (1964), Vincent (1960), and others indicate that physiological differences may exist.

Materials and Methods

The study was based on 422 active and 196 standard metabolism measurements from October 1966 to December 1967. Hatchery (Hull-Erickson) and wild (De Smet) trout were raised in hatcheries of the Utah State Division of Fish and Game at Logan and Glenwood, respectively. At the hatcheries, fish were held outdoors and exposed to natural photoperiods. Fish were fed a commercial pellet fish food. They weighed approximately 100–400 g and were 10–14 months old. Fish were acclimated to 5, 10, 15, 20, and 25 C. They survived well at the lower temperatures, but at 25 C they refused to feed and were generally smaller than others.

One fish was used in each measurement of metabolism. Fish were starved for 24 hr before transfer to the respirometers to avoid undue deposition of feces in the chambers. After each experiment, fish were killed, dried with ab-

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[1] Based on a thesis submitted by I.W.D. in partial fulfillment of the requirements for the degree of Doctor of Philosophy from the Department of Wildlife Resources, Utah State University, Logan, Utah.

[2] Present address: Department of Mines and Natural Resources of Manitoba, Winnipeg, Man.

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sorbent paper, weighed, measured (fork length), and sexed.

Unless otherwise indicated, all experiments were conducted under continuous laboratory illumination (2–12 ft-c). Water analyses except those for temperature and dissolved oxygen were done by the Utah Water Research Laboratory (Table 1). The Alsterberg azide modification of the Winkler method (American Public Health Association 1960) was used to determine dissolved oxygen concentration. The difference in dissolved oxygen concentration in water samples collected from the respirometers at the beginning and end of each test period, multiplied by the respirometer volume, was taken as the amount of oxygen consumed by a fish. On the few occasions that two oxygen determinations on a water sample differed by more than 0.1 ml sodium thiosulphate (0.01 N), a third titration was done and the three measurements averaged.

"Air saturation" in this study was taken as dissolved oxygen concentrations in excess of 90% air saturation. The duration of the test periods was varied according to the water temperature to maintain relatively high dissolved oxygen concentrations, those at the end of tests being seldom less than 6 ppm and rarely as low as 5 ppm. The decline in concentration during the test period due to fish respiration was usually about 1.0 ppm and seldom exceeded 2.0 ppm.

TABLE 1. Physical and chemical properties of water from fish acclimation tanks, Utah State University Fisheries Laboratory, Logan, Utah, 1967. Expressed as parts per million unless stated otherwise.

Item	Mean	Range
Color (*units*)	<5	–
Turbidity (*JTU*)[a]	<25	–
Dissolved oxygen	8.6	6.7–10.0
Temp (*C*)	–	5–25
Total hardness as $CaCO_3$	187	160–210
Alkalinity total	216.2	212.2–218.3
Bicarbonate	216.2	212.2–218.3
Carbonate	0	–
Chloride as Cl	5.6	3.5–7.5
Calcium as Ca	18.0	5.3–35.2
Magnesium as Mg	33.9	23.6–41.8
Sodium as Na	1.4	1.2–1.8
Potassium as K	0.4	0.3–0.6
Sulphate as SO_4	8.36	6.70–9.60
Ammonia as NH_3	0.44	0–0.78
Phosphate as PO_4	0.189	0.005–0.340
Nitrate as NO_3	0.40	0–1.00
Silica as SiO_2	3.4	2.9–3.8
Electrical conductivity (*µmhos/cm*)	311	290–325
Total dissolved solids	180	158–207
*p*H	8.2	7.4–8.8

[a] Jackson Turbidity Units.

RESPIROMETERS

Respirometers were submerged in 400-liter constant-temperature water baths. Water temperatures in the baths were controlled by buffering thermoregulator-controlled heaters against a Min-O-Cool[3] refrigeration unit, in the active respirometer bath within ±0.3 C, and in the standard respirometer bath within ±0.1 C.

Active metabolic rates were determined using a 27.5-liter respirometer slightly modified from apparatus described by Smit (1965) and Blazka et al. (1960). It consisted of two clear acrylic plastic chambers in which water was drawn through the inner cylinder by a propeller, and returned between the outer and inner cylinders. Fish were forced to swim against the current in the inner cylinder. A stainless-steel grid in front of the propeller, electrified with approximately 8–10 v a-c, stimulated the fish to continue swimming. Fish were inserted and and removed through a lid in the top.

Standard metabolism measurements were made with apparatus resembling that described by Beamish and Mookherjii (1964). A 12.6-liter, thin-walled, stainless-steel sauce pot fitted with a flowmeter was used for the respiration chamber. The flowmeter operated on a heat-loss principle and was used to estimate the activity of a fish in the chamber. This respirometer differed from that employed by Beamish and Mookherjii (1964) in several aspects: this chamber increased conductance of heat to the outside bath; time of heater operation was measured in seconds by a manual reset electrical timer; variation in baseline settings was reduced by adjusting the heater circuit closure time to 50% (±3%) of the experimental period; water samples were collected manually; the thermoregulator was installed directly into a simple heater relay-coil circuit; and the respirometer was flushed at a rate of approximately 1.5 liters/min by an electric pump. Fish in the darkened chamber appeared indifferent to the introduction, presence, or removal of the pump-flushing tube.

ACCLIMATION

Procedures described by Beamish and Mookherjii (1964) were followed for acclimating fish to 5, 10, 15, 20, or 25 C. For each temperature, 25 fish were placed in an insulated 400-liter tank. The water exchange was maintained at 115 liters/hr. Water temperatures in the tanks were increased or decreased 1 degree C per day until the desired temperature was obtained. Fish were held at the test temperatures for at least 2 weeks before they were used in experiments. Acclimation temperatures were controlled to within ±0.5 degree C.

ACTIVE METABOLISM

Immediately before active metabolism measurements, each fish was allowed a 15–20-min adjustment period in the respirometer while it was flushed to allow dissolved oxygen concentrations to reach air saturation. During this time, water was circulated slowly in the respirometer to allow each new fish to orient itself. Fish were usually exercised for 15–20 min at a velocity of about 91 cm/sec (3.0–4.7 lengths/sec) depending on the temperature. Temperatures of 5 and 25 C reduced swimming ability of some trout and these fish were exercised at lower velocities (67 cm/sec) at these temperatures.

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[3] A refrigeration unit and fiberglass reservoir.

Preliminary tests were conducted to determine the approximate velocities required to cause fish of different sizes acclimated to different temperatures to respire at their active metabolic rate. Seventy small (60–147 g) and 55 large (135–241 g) hatchery trout were acclimated to 5, 10, 15, 20, and 25 C and exercised at velocities from 43 to 107 cm/sec (1.5–5.1 lengths/sec). Where possible, at least two measurements of oxygen consumption were made for small and large fish at each velocity and temperature. Results indicated that the active metabolic rate was not significantly increased at velocities higher than 67 cm/sec (Fig. 1). These tests indicated that in some instances, fish may respire at active metabolic rates when forced to swim at speeds as low as 1.5 lengths/sec (41 cm/sec). This is not remarkable because fish stimulated by handling immediately before experiments (Keys 1930; Wells 1932; Black et al. 1939) or by an electric field (Basu 1959) may respire at the active rate at low water velocities. However, the mere handling of fish is not always sufficient to induce maximum oxygen consumption, and fish must be forced to swim and exert themselves to obtain this value (Job 1955). It was concluded, therefore, that handling of fish, the electrical stimulus, and forced exercise at relative high swimming speeds were sufficient for full expression of the active metabolic rate in these experiments.

STANDARD METABOLISM

Before standard metabolism experiments, a baseline reading for the flowmeter heater was obtained immediately before placing a fish in the respirometer. Each fish was allowed an overnight period of adjustment to the respirometer. A black plastic cover was fitted over the transparent lid of the chamber and precautions were taken (Fry 1957) to suppress external stimuli that might stimulate the fish. Fresh water was pumped through the chamber (1.5 liters/min) continuously before each experiment. Oxygen consumption and activity determinations lasted 40 min at 5 C; 30 min at 10, 15, and 20 C; and 15 min at 25 C. At the end of each test period, activity was recorded, and the chamber flushed for 20 min to restore the original concentration of dissolved oxygen.

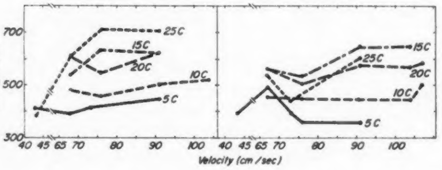

FIG. 1. Active metabolism of hatchery rainbow trout in two size ranges at various water velocities and temperatures.

Each experiment lasted 5–7 hr to allow at least six simultaneous determinations of oxygen consumption and spontaneous activity. Results were discarded when baseline determinations before and after each experiment differed by more than 60 sec (6.7% of the shortest test period). The six determinations of oxygen consumption and activity were plotted on a semilogarithmic grid. A straight-line relation was obtained by plotting the logarithm of oxygen consumption against activity. Standard metabolism was estimated by extrapolation of this line to zero activity.

The semilogarithmic relation between oxygen consumption and activity presumably resulted from the high degree of swimming activity permitted in the large respirometers used in this study. Using the same type of flowmeter and relatively smaller respirometers, McKenzie (MS 1960), Beamish and Mookherjii (1964), and Mather and Shrivastava (MS 1967) found a linear arithmetic relation between oxygen consumption and spontaneous activity. Beamish and Dickie (1967) attribute the comparatively high rates of oxygen consumption of fish spontaneously active at low levels of activity to the high cost of intermittent swimming, which becomes more efficient at faster, more continuously maintained speeds.

Unlike measurements of active metabolic rates, standard metabolism measurements of rainbow trout were subject to considerable variation. Some experiments involved "excited" or "nervous" fish that exhibited high levels of activity. Prolonged habituation of the fish to the chamber, exclusion of light from the chamber, and other precautions taken to reduce the fish to a quiescent state were unsuccessful. Smit (1965) and Brett (1964, 1965) discussed how such behavioural characteristics as excitability, restlessness, and spontaneous movement can contribute to the variability of estimates of standard metabolism. Beamish and Dickie (1967) considered that this variability in behaviour was mainly responsible for the "unavoidably high experimental error" usually associated with estimates of standard oxygen consumption.

STARVATION AND CONTROLLED LIGHTING

Hatchery trout acclimated to 15 C were starved for 1, 2, 3, 6, and 9 days. Fish were forced to swim at 91 cm/sec velocity (3.0–4.0 lengths/sec) except that those starved for 9 days were unable to swim at the higher velocities and were exercised at 67–91 cm/sec velocities (2.5–3.6 lengths/sec). Active and standard metabolism rates of starved fish were measured from 7:00 AM to 5:00 PM and from 7:00 AM to 2:00 PM, respectively.

Active and standard metabolic rates were determined using wild trout subjected to continuous light (2–12 ft-c) and to light controlled to coincide with natural day length. A light-proof enclosure was erected around two acclimation tanks, and the photoperiod adjusted to coincide with natural length of day. Fish were exposed to periods of different light intensities in succession as follows: 30 min of 28–60 ft-c, 9 hr of 380–450 ft-c, 30 min of 28–60 ft-c, and 14 hr of darkness. The effect of light on active and standard metabolism was investigated during morning (7:00 AM–1:00 PM) and afternoon (1:00 PM–7:00 PM).

STATISTICAL TREATMENT

Weights of fish were adjusted by covariance analysis to a common mean and, unless otherwise indicated, these and other statistical results of active and standard metabolism experiments are reported at $P = 0.05$. In some instances, statistical analyses without covariance were employed to compare scope for activity values, and standard and active metabolic rates. However, these respiratory rates were compared only when mean weights of fish were not significantly different at $P = 0.05$. Based on the z distribution described by Ostle (1963), respiratory rates were significantly different at $P = 0.05$ if the calculated z value exceeded 1.96.

Results and Discussion

EFFECTS OF TEMPERATURE

Scope for activity

Scope for activity increased from 348 and 324 mg O_2/kg per hr at 5 C to a maximum of 498 mg O_2/kg per hr at 15 and 20 C for hatchery and wild trout, respectively (Table 2). At 25 C, scope for activity was unchanged for wild trout, but was significantly decreased for hatchery trout. Lowest scope for activity for hatchery and wild trout occurred at 25 and 5 C, respectively. Scope for activity of hatchery trout was less at 5 C than at 10, 15, or 20 C ($z \geqslant 4.03$) and lower at 10 C than 15 and 20 C ($z \geqslant 2.30$). Similarly, scope for activity of wild trout was less at 5 C than at 10 and 15 C ($z \geqslant 4.38$), and was less at 10 C and 20 C ($z = 2.03$). Hatchery and wild trout acclimated to the lower temperatures weighed significantly more than those at higher temperatures, and this prevented statistical comparison of scope for activity between these temperatures. However, in accordance with Job's findings (1955) any increase in mean weight of fish acclimated to higher temperatures would tend to further decrease scope for activity at these higher temperatures. Job (1955) studied the effect of weight on respiratory metabolism of brook trout (*Salvelinus fontinalis*) and found that the rate of increase in scope for activity with rising temperatures was progressively reduced for larger fish.

Scope for activity was similar for hatchery and wild trout at each temperature except 25 C, at which it was significantly higher ($z = 3.88$) for wild trout. This markedly different response at 25 C suggests that wild trout may be the more active in warm water. Increased activity of wild trout would be expected, as increased scope for

TABLE 2. Active and standard metabolism and scope for activity (mg O_2/kg per hr) of hatchery and wild rainbow trout at five temperatures. Values (active and standard rates only) joined by underline are not significantly different from each other at $P = 0.05$ (Newman-Keuls multiple range test).

Trout	Date (*1967*)	No. tests[a]	Wt (*g*)	Length (*mm*)	Swimming speed (*lengths/sec*)	Temp (*C*)				
						5	10	15	20	25
					Active metabolism					
Hatchery	Apr. 22–June 24	54	137–249	230–285	2.3–3.8	384(15)[b]	468(11)	576(22)	570(22)	478(18)
Wild	Sept. 28–Oct. 30	65	79–398	198–300	2.9–4.7	366(16)	492(20)	552(37)	592(19)	588(19)
					Standard metabolism					
Hatchery	Apr. 17–June 29	48	148–264	235–290	–	36(2)	42(5)	78(6)	84(7)	138(12)
Wild	Sept. 25–Oct. 27	55	85–396	208–318	–	42(2)	54(4)	60(5)	94(10)	120(18)
					Scope for activity					
Hatchery						348(16)	426(21)	498(37)	486(20)	336(23)
Wild						324(16)	438(20)	492(37)	498(22)	468(26)

[a] Number of tests at each temperature ranged from 8 to 15 and averaged 12.

[b] Numerals in parentheses represent one standard error of the mean.

activity indicates more "available energy" for swimming (Brett 1964). This additional energy available to wild trout may be an important survival mechanism during times of stress. If scope for activity may be used to assess environmental stress as Brett (1958) has suggested (a decreased scope indicating stress conditions), wild trout in water of high temperature may be better able to cope with temperature stress and have greater potential for survival than hatchery trout under these conditions.

The difference in scope for activity between hatchery and wild trout may be attributed to differential effects of hatchery and natural selection on their genetic compositions. Hatchery trout were produced from brood stock maintained in Utah hatcheries, and had experienced hatchery selection since 1880 (Dollar and Katz 1964). Eggs for the wild stock were obtained from De Smet Lake, Wyoming, where wild trout have been exposed to natural selection processes since as early as 1912. Differences observed by Flick and Webster (1964) and Smith (1957) in behaviour, growth, and survival of hatchery and wild trout suggest genetic implications. Unlike hatchery trout maintained in water of relatively constant temperature, wild trout exposed to the temperature extremes and fluctuations encountered in nature may have adapted to high water temperatures.

Active metabolism

Temperature had a significant effect on active metabolic rates of hatchery trout ($P = 0.01$; $F = 20.05$) and wild trout ($P = 0.01$; $F = 23.70$). Highest active metabolic rates for hatchery and wild trout occurred at 15 and 20 C, and were 576 and 592 mg O_2/kg per hr, respectively. Active rates for hatchery trout at 5 and 10 C were significantly lower than those at other temperatures, and active metabolism of wild trout was significantly different at 5, 10, 20, and 25 C (Table 2). Active metabolism of wild trout was significantly higher than active metabolism of domestic trout at 25 C ($z = 4.35$) and this accounted for the higher scope for activity for wild trout at 25 C. Mean rates of active metabolism of hatchery and wild trout did not differ significantly at other temperatures ($z \overset{=}{<} 1.05$).

The decrease in active metabolic rates of hatchery trout at temperatures greater than 15 C may be explained by lowered dissolved oxygen concentrations at these higher temperatures. This, however, does not acount for the difference in active metabolism between hatchery and wild trout at 25 C. Active metabolic rates of wild trout at 25 C were not significantly lowered at 20 C. Similarly, Brett (1964) found no statistical difference between active metabolic rates of sockeye salmon (*Oncorhynchus nerka*) acclimated to 15, 20, and 24 C, although the mean rates tended to decrease at temperatures above 15 C. He attributed this decline in active metabolism of sockeye salmon at high temperatures to the reduced oxygen available in solution at high temperatures, and "other factors of a physiological nature."

Standard metabolism

Temperature had a significant effect on standard metabolism of hatchery trout ($P = 0.01$; $F = 17.65$) and wild trout ($F = 6.17$). Standard metabolic rates of hatchery trout at 5 and 10 C were significantly lower than those at higher temperatures and were significantly higher at 25 C than at other temperatures (Table 2). Standard metabolic rates of wild trout at 5, 10, and 15 C were significantly lower than those at higher temperatures. Standard metabolism increased progressively from a low of 36 and 42 mg O_2/kg per hr at 5 C to a high of 138 and 120 mg O_2/kg per hr at 25 C for hatchery and wild trout, respectively. Standard metabolic rates of hatchery and wild trout showed similar increases with temperature except at 15 C, at which standard metabolic rates of hatchery trout were higher ($z = 2.29$).

In standard metabolism measurements, the relation between water temperature and the logarithm of oxygen consumption rate was linear for hatchery and wild trout over the range of temperatures investigated (Fig. 2). Least squares fitted to the points were: $\log Y = 1.388 + 0.029\ X$ and $\log Y = 1.492 + 0.023\ X$ for hatchery and wild trout respectively, where $Y = \log_{10}$ oxygen uptake rate (mg O_2/kg per hr) and $X =$ water temperature (C).

The semilogarithmic relation between standard oxygen consumption and temperature for hatchery and wild rainbow trout differs somewhat from the slightly convex curvilinear relation reported for warmwater species (Beamish 1964c), but appears

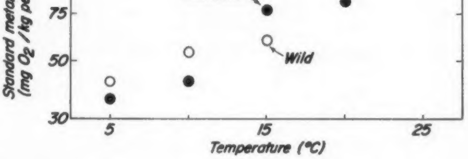

FIG. 2. Relation of temperature and standard metabolism of hatchery and wild rainbow trout.

typical for cold-water species (Gibson and Fry 1954; Beamish 1964c; and Brett 1964).

Temperature increased the ratios of active and standard metabolic rates approximately 9–11 times at 15 C, and decreased these ratios to three to five times the standard rate at 25 C for hatchery and wild trout, respectively. The maximum active rate was about 16 times the lowest standard rate. Brett (1964) reported similar ratios to describe the effect of temperature on active and standard metabolic rate of sockeye salmon.

EFFECTS OF STARVATION

Scope for activity

Starvation increased scope for activity of hatchery trout from a low of 414 mg O_2/kg per hr at 1 day's starvation to a high of 486 mg O_2/kg per hr after 6 days' starvation. Scope decreased at 9 days' starvation to 456 mg O_2/kg per hr (Table 3). Although mean active metabolic rates were not significantly different for different levels of starvation, these individual means were used in preference to a common mean in calculations of scope for activity. Scope for activity at 6 days' starvation differed significantly from that at 1 and 3 days' starvation ($z \geq 2.42$). The increased scope for activity after starvation for 6 days suggests that more oxygen may be available for metabolic functions that demand maximum energy.

Active metabolism

Starvation had no significant effect on rates of active metabolism of hatchery trout. Mean active rates of oxygen consumption ranged from a low of 498 mg O_2/kg per hr after 3 days' starvation to a high of 558 mg O_2/kg per hr after 6 days' starvation (Table 3).

The effect of starvation on active metabolism of fish has received little attention. Fry (1957) reported results obtained by Barrett who investigated the effect of starvation on active metabolism of rainbow trout. Fish were maintained at approximately 11 C and were starved for 100 days. Barrett found "no essential difference" between the active rates of starved and control animals.

Standard metabolism

Standard metabolic rates of hatchery trout decreased after 2 days' starvation but increased slightly with continued starvation (Table 3). Highest standard oxygen consumption was 96 mg O_2/kg per hr after 1 day's starvation, and the lowest 60 mg O_2/kg per hr after starvation for 2 days. Standard metabolism differed significantly between 1 and 2 days' starvation (F = 3.42).

Beamish (1964a) reported a decrease in standard metabolism of fish during the first 2 or 3 days of starvation, and he attributed this decrease to the reduced oxygen required for assimilation of food. A decreased oxygen requirement for food assimilation would explain the significant reduction in standard metabolism of rainbow trout after 2 days' starvation.

EFFECTS OF DAYLIGHT HOURS

Scope for activity

Scope for activity of wild trout maintained under controlled conditions of light and dark coinciding

TABLE 3. Active and standard metabolism and scope for activity of starved hatchery rainbow trout at 15 C.

Date (*1967*)	No. tests[a]	Wt (*g*)	Length (*mm*)	Swimming speed (*lengths/sec*)	Starvation period (*days*)				
					1	2	3	6	9
				Active metabolism					
July 6–25	50	146–328	230–255	2.5–4.0	510(21)[b]	510(12)	498(23)	558(16)	528(17)
				Standard metabolism					
June 28–Aug. 14	45	150–311	235–305	–	96(5)	60(7)	84(7)	72(7)	72(5)
				Scope for activity					
					414(22)	450(14)	414(24)	486(17)	456(18)

[a] Number of tests for each starvation period ranged from 9 to 10.

[b] Numerals in parentheses represent one standard error of the mean.

with the natural photoperiod was 450 ± 20 mg O_2/kg per hr in the forenoon and 480 ± 25 mg O_2/kg per hr in the afternoon. Scope for activity of fish maintained under constant light was 456 ± 25 mg O_2/kg per hr in the forenoon and 462 ± 25 mg O_2/kg per hr in the afternoon. Forenoon and afternoon periods had no significant effect on scope for activity.

There appears to be no general agreement concerning the existence of diurnal differences in the metabolic rate of fish (Winberg 1956). In the present study, the absence of differences in respiratory metabolism of wild rainbow trout during the daylight hours suggests that any diurnal differences must occur at night.

Active and standard metabolism

Active and standard metabolic rates of wild trout were not significantly different (F = 0.74) between forenoon and afternoon periods (Table 4). Endogenous daily cycles of activity during the diurnal period may affect respiratory metabolism measurements, particularly standard metabolism measurements, in spite of maintenance of constant conditions (Fry 1957). In the present study, however, the influence of spontaneous activity on oxygen consumption was quantified and eliminated from estimates of standard metabolism of rainbow trout and could not contribute to diurnal differences in the metabolic rate. Clausen (1933, 1936), Oya and Kimata (1938), and Graham (1949) described diurnal influence on fish metabolism by measuring oxygen consumption throughout a 24-hr period. The influence of spontaneous activity on oxygen consumption was not determined, although precautions were taken to reduce external stimuli and induce the fish to a quiescent state. Higginbotham (1947) found that the respiratory metabolism of channel catfish (*Ictalurus punctatus*) was higher in the afternoon than in the morning and attributed this to increased spontaneous activity during the afternoon.

EFFECTS OF SEASON AND SEX ON ACTIVE METABOLISM

Ten to 49 measurements of active metabolism of mature hatchery rainbow trout acclimated to 15 C were made on five separate occasions: October–November 1966, and February, April, July, and August 1967 (Table 5). This provided an opportunity to investigate seasonal influence on active rates of oxygen consumption and seasonal effects on active metabolism of the sexes.

Season

For males, the minimum active rate of oxygen consumption was 546 mg O_2/kg per hr during October–November 1966, and increased to a high of 618 mg O_2/kg per hr in February 1967. Active rates remained relatively high in April, but decreased to a low of 522 mg O_2/kg per hr in July. Mean active metabolic rates for the five test periods were not significantly different (F = 1.38).

TABLE 4. Active and standard metabolism of wild rainbow trout at 15 C during forenoon and afternoon periods of controlled lighting.

Light conditions	Daytime period	Wt (*g*)	Length (*mm*)	Swimming speed (*lengths/sec*)	Mean metabolic rate (*mg O_2/kg per hr*)
		Active metabolism			
Controlled light and dark[a]	Forenoon	153–261	240–292	3.1–4.3	534(19)[c]
	Afternoon[b]	88–247	195–328	3.1–5.3	558(24)
Continuous light (*2–12 ft-c*)	Forenoon	86–325	190–306	2.5–4.8	528(24)
	Afternoon	100–576	210–360	2.5–4.8	534(23)
		Standard metabolism			
Controlled light and dark[a]	Forenoon	110–306	230–297	–	84(7)
	Afternoon	86–309	200–296	–	78(8)
Continuous light (*2–12 ft-c*)	Forenoon	105–381	217–310	–	72(6)
	Afternoon	116–330	220–305	–	72(9)

[a] Light intensities in succession as follows: 30 min of 28–60 ft-c, 9 hr of 380–450 ft-c, 30 min of 28–60 ft-c, and 14 hr of darkness.

[b] Eleven fish used in this test, and 12 in each of the others.

[c] Numerals in parentheses represent one standard error of the mean.

TABLE 5. Active metabolism of hatchery rainbow trout at 15 and 10 C in relation to season and sex.

	Wt (*g*)	Length (*mm*)	Swimming speed (*lengths/sec*)	1966	1967			
				Oct.–Nov.	Feb.	Apr.	July	Aug.
Males (15 C)	96–340	192–303	2.7–4.4	546(28)[a]	618(22)	588(30)	522(38)	558(14)
Females (15 C)	92–342	202–305	2.6–4.5	492(10)	600(29)	570(32)	498(24)	528(29)
Males (10 C)	106–321	210–288	3.1–4.4	462(15)	–	–	–	–
Females (10 C)	143–354	230–297	3.1–4.0	408(18)	–	–	–	–

[a] Numerals in parentheses represent one standard error of the mean.

For females, the seasonal trends in active metabolism were similar to those for males. Mean active rates of oxygen uptake increased from a minimum of 492 mg O_2/kg per hr in October–November 1966, to a maximum of 600 mg O_2/kg per hr in February 1967.

The effect of season upon active rates of oxygen consumption of females was significant (F = 3.18). Active metabolic rates in October–November were significantly lower than those in February and April. Highest active metabolic rates of male and female rainbow trout occurred during the seasonal period associated with spawning (February).

Although the influence of season on active metabolism has received little attention, seasonal effects on standard oxygen consumption have been studied by Wells (1935), Privolnev (1948), and Beamish (1964b). Increased respiration rates of fish species at various times of the year appear to be related to the reproductive cycle of each particular species. Wohlschlag and Juliano (1959) found that respiration rates (undifferentiated) of swimming bluegill (*Lepomis macrochirus*) were higher in winter than in the spring, summer, and autumn seasons, and they suggested that adaptation to cold temperature accounted for the higher metabolic rates during the winter months.

Sex

Active metabolic rates of males were greater than those of females during the five seasonal periods (Table 5). The relative difference in all instances was 3–10% greater than the female rate. Since the mean weights of males and females were not significantly different, the mean active rates for the sexes were compared at each season. Active rates were significantly higher for males than for females during October–November 1966 and August 1967 ($P = 0.10$; $z = 1.82$ and 1.71). Using covariance analyses, however, highly significant differences were found during October–November 1966 when active rates of males at 15 C were higher than those of females ($P = 0.01$; $F = 24.1$). Similarly, at 10 C males had significantly higher active rates than females ($P = 0.06$; $F = 4.11$) during October–November 1966.

The consistently higher active metabolic rates observed for males and the statistically significant results obtained, particularly from covariance analyses, provide considerable evidence in support of the hypothesis that males have higher active metabolic rates than females. Although the effect of sex on active metabolism has not been previously reported, Wohlschlag and Juliano (1959) found no significant difference in routine metabolic rates (Fry 1967) of male and female bluegill, although rates of oxygen consumption were consistently higher for females. They suggested that any effect of sex could better be established by confining experiments to more limited environmental fluctuations. Beamish (1964b) found no difference in standard rates of oxygen uptake for male and female brook trout at different seasons. Similarly, he found no difference in standard rates between the sexes for brown trout (*Salmo trutta*) during different seasons, with the exception of the period September–November when standard rates of males were considerably higher than those of the females. He suggested that the males may have shown a seasonal response whereas females did not.

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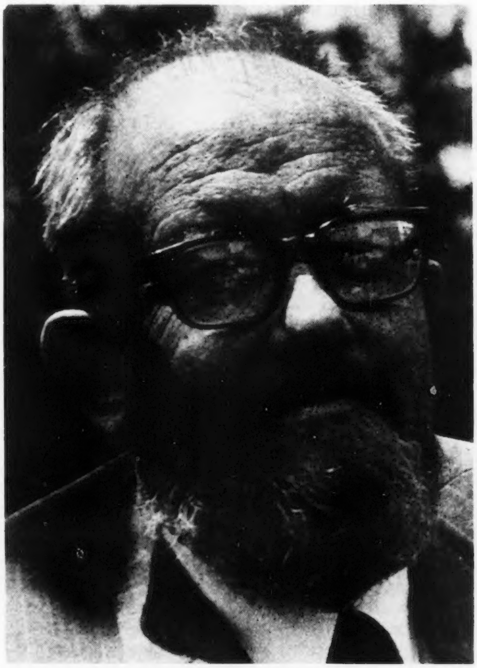
John Douglas Hipwell Strickland
1920-1970

Dr J. D. H. Strickland died of kidney failure on November 12 in San Diego, California.

He was born in London, England, on August 3, 1920, and received his academic training from the University of London. He worked in England for the Ministry of Supply and was later a senior scientific officer with the U.K. Atomic Energy Authority. Dr Strickland emigrated to Canada in 1952 and was first employed by the British Columbia Research Council. He became a Canadian citizen and in 1956 he started his career in marine science as a senior scientist with the Pacific Oceanographic Group of the Fisheries Research Board of Canada. In this field of work Dr Strickland very quickly laid the basis for a renaissance in chemical methodology and primary production studies in the sea. His most notable contribution after only two years in marine research was his paper on light attenuation (J. Fish. Res. Bd. Canada 15: 453–493, 1958). Two bulletins appeared in 1960 (Fish. Res. Board Can. Bull. 122 and 125); the former, on primary production, is an accepted text among scientists today, and the latter has undergone three revisions to meet a widespread demand for a text on seawater analysis. Among other activities during 10 years of work with the Fisheries Research Board, Dr Strickland undertook a large-scale underwater experiment in which over 30,000 gallons of sea water were enclosed for the purpose of carrying out detailed studies on mechanisms leading to the primary formation of food in the sea.

In 1966 he became head of a new Marine Food Chain Group of the University of California's Institute of Marine Resources. The Institute is located on the campus of San Diego's Scripps Institution of Oceanography, where Dr Strickland also served as a lecturer. The Marine Food Chain Group quickly grew into one of the most formidable assemblages of marine scientists in the world. Dr Strickland's own particular research interests as head of the group included studies on the chemistry of seawater nutrients, the kinetics and chemical composition of phytoplankton, the role of bacteria, detritus, and dissolved organic matter in the marine food web, and zooplankton trophodynamics. He investigated the local Californian red-tide phenomenon and the diurnal vertical migration of phytoplankton in a specially constructed deep outdoor tank.



Dr Strickland served as a panel member on the Committee on Oceanography of the National Academy of Sciences and also as a member of the Chemistry Panel of the American International Union of Geodesy and Geophysics. He was a member of the American Society of Limnology and Oceanography, the American Institute of Biological Sciences, and the American Association for the Advancement of Science. He was a Fellow of the Royal Institute of Chemistry of Great Britain and Ireland, of the Chemical Society of London, and of the Chemical Society of Canada.

John's ambition took him from a London grammar school to one of the greatest academic institutions in the world. At each turning point in his career he left behind him a job well done and many friends who will remember his guidance, lively debate, and obsession with hard and thorough work. He was a leader among men and he earned their respect. It was a privilege to have worked with him.

Dr Strickland is survived by his wife Muriel, two sons, Robert and Alec, and a daughter, Helen.

T. R. Parsons

NOTES

Investigation of the Fatty Acid Composition of Oils and Lipids from the Sand Launce (*Ammodytes americanus*) from Nova Scotia Waters

R. G. Ackman and C. A. Eaton

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Ackman, R. G., and C. A. Eaton. 1971. Investigation of the fatty acid composition of oils and lipids from the sand launce (*Ammodytes americanus*) from Nova Scotia waters. J. Fish. Res. Bd. Canada 28: 601–606.

A commercial oil from sand launce (*Ammodytes americanus*), and triglyceride fractions from lipids of two samples of fish also from Nova Scotia waters, showed essentially the same fatty acid composition as herring oils, indicating that commercial utilization of sand launce oil would be practical. Minor differences in the sand launce oil fatty acids included about the same levels of 20:1 and 22:1, and a somewhat higher figure for 16:1. Comparisons are also made with capelin oil and sand launce oils from Danish production.

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The sand launce, *Ammodytes americanus* (= *A. hexapterus*, alternatively *A. dubius* if considered the offshore or "northern" sand launce; see also Leim and Scott 1966; Scott 1968; Winters 1970) has been considered as a potential Canadian fishery resource for reduction to meal and oil (Scott 1969).

Exploratory fishing for sand launce was carried out by the Nova Scotia Department of Fisheries in 1968 (Jones 1968), and we were fortunate enough to secure a sample of oil prepared from commercial reduction of one of their catches. To further our knowledge of the potential of this species for commercial utilization we secured intact fish in 1970 and have separated and examined the two principal lipids, triglycerides and phospholipids, the former essentially representing a commercial oil. Fatty acid composition studies are reported for comparison with data for North Sea sand launce, *Ammodytes tobianus* (Hølmer 1967), for the related Japanese "sand eel," *Ammodytes personatus* (Ueda and Nagaok 1968), and with locally produced oils from the Atlantic herring, *Clupea harengus harengus*.

Materials and methods — A commercial sand launce oil sample was obtained from fish taken off the Emerald Bank by the M/V *Whispering Sea* and reduced June 14, 1968, at the British Columbia Packers' plant in Lower East Pubnico, N.S. The oil yield was 4% of the fish (Jones 1968). The oil, Wijs iodine value (IV) 143.0, was saponified, and the unsaponifiable materials (2.4%) were removed by method Ca-6b-53 of the American Oil Chemists' Society. The fatty acids were recovered and converted to methyl esters with BF_3–MeOH reagent.

On April 29, 1970, the *Whispering Sea* obtained sand launce on the West Sable Island Banks. The fish were iced until landed in Halifax on April 30, where they were frozen and stored at –40 C. On August 12, 1970, 21 whole fish were extracted by the method of Bligh and Dyer (1959). Records for these fish were not kept, but six other fish from the same lot averaged 40.2 g in weight and 24.8 cm in length.

On July 7, 1970, the M/V *Harengus* obtained a few sand launce on the Middleground Banks, approximately 100 miles east of Halifax. These were iced, landed in Halifax on July 8, and frozen and stored at –40 C until August 4. Three fish were extracted as described above, and five were minced and boiled briefly to liberate mechanically a small amount of oil. The average weight of these eight fish was 42.3 g.

The lipid extracts were chromatographed on a column of divinylbenzene copolymer beads as described by Sipos and Ackman (1968) to yield phospholipid and triglyceride fractions. Iodine values were determined for total lipids and on the isolated triglycerides (Table 1). Methyl esters were prepared with BF_3–MeOH from phospholipids and triglycerides by transesterification, from the fatty acids recovered from total lipids after removal of unsaponifiable materials, and from total lipid (July 8) by transesterification.

Gas–liquid chromatographic examination of the fatty acids was carried out on open-tubular columns, 150 ft (50 m) in length and 0.01 inch (0.25 mm) in internal diameter, coated with butanediol–succinate polyester.

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TABLE 1. Composition (weight percent) of methyl esters of fatty acids derived from sand launce oil and lipid fractions. Iodine values for methyl esters are also given.

		Lipids, 1970					
	Commercial oil, 1968,	Triglyceride		Total	Phospholipid		North Sea sand launce
Fatty acid	June	April	July	July	April	July	oil, 1966[a]
12:0	0.20	0.24	0.17	0.15	0.04	0.54	–
Iso-14:0	0.09	0.04	0.04	0.06	0.17	0.25	–
14:0	7.67	7.98	7.39	6.70	2.14	2.15	4.0
4,8,12-TMTD[b]	0.20	0.36	0.25	0.21	0.16	0.09	
Iso-15:0	0.47	0.42	0.37	0.44	0.09	0.06	0.3
Anteiso-15:0	0.12	0.19	0.15	0.13	0.03	0.04	
15:0	0.47	0.55	0.50	0.48	0.32	0.39	0.4
Iso-16:0	0.09	0.27	0.12	0.07	0.05	0.21	0.3
2,6,10,14-TMPD[b]	0.23	0.07	0.03	0.07	0.05	0.03	
16:0	12.90	16.75	12.53	13.14	23.56	23.26	19.7
Iso-17:0	0.07	0.27	0.12	0.18	0.26	0.08	0.4
Anteiso-17:0	0.03	0.09	0.11	0.11	0.16	0.06	
17:0	0.24	0.45	0.18	0.43	0.32	0.34	0.5
3,7,11-15-TMHD[b]	0.17	0.13			0.10	0.17	–
Iso-18:0	0.21	0.07	0.10	0.10	0.18	0.22	0.2
18:0	0.81	1.25	0.85	0.93	4.66	3.59	2.5
19:0	0.08	0.04	0.12	0.10	0.05	0.03	–
20:0	0.08	0.38	0.07	trace	0.04	0.04	0.2
Total saturated acids	24.13	29.55	23.10	23.30	32.38	31.55	28.5
14:1	0.09	0.13	0.10	0.15	0.01	0.10	0.1
15:1	0.07	0.13	0.12	0.07	0.01	0.17	–
16:1ω9	0.28	0.18	16.00	0.21	0.13	0.27	7.3
16:1ω7	12.67	11.00		14.15	2.79	2.92	
16:1ω5	0.59	0.18	0.57	0.51	0.08	0.35	
17:1ω8	0.17	0.18	0.10	0.14	0.13	0.24	0.3
18:1ω11 & 9	5.35	7.33	4.60	4.85	8.27	6.32	9.2
18:1ω7	1.72	2.47	1.90	2.34	2.04	3.01	
18:1ω5	0.40	0.66	0.62	0.78	0.62	0.51	
19:1	trace	0.11	0.09	0.04	0.02	trace	0.1
20:1ω11 & 9	12.36	7.22	12.20	11.42	2.70	2.77	10.8
20:1ω7	0.33	0.44	0.76	0.59	0.12	0.14	
20:1ω5	0.02	0.09	0.06	0.09	0.07	–	–
22:1ω13 & 11	12.89	7.81	14.19	12.36	2.52	1.74	12.8
22:1ω9	0.84	0.76	1.41	1.03	0.35	–	
22:1ω7	0.28	0.16	0.31	0.59	0.15	–	
24:1ω9	1.10	0.51	0.68	0.95	1.73	0.83	0.7
Total monounsaturated acids	49.16	39.36	53.71	50.27	21.74	19.37	41.3
16:2ω6	0.07	0.13	0.02	0.14	0.46	0.20	0.4
16 ?	0.04	0.58	1.07	0.97	0.72	0.52	
16:2ω4	0.81				–	–	–
16:3ω4	0.67	0.62	0.68	0.64	0.05	0.14	1.0
16:3ω3	0.02	0.06	0.07	trace	–	–	–
16:4ω3	0.06	0.08	0.08	0.18	0.02	–	–
16:4ω1	1.35	1.59	1.14	1.42	–	–	0.1
18:2ω6	0.79	1.04	1.04	1.00	1.61	1.40	0.6
18:2ω4?	0.17	0.29	0.10	0.15	0.08	trace	0.1
18:3ω6	trace	0.09	–	0.06	0.05	0.03	0.1

(*Continued*)

TABLE 1. Composition (weight percent) of methyl esters of fatty acids derived from sand launce oil and lipid fractions. Iodine values for methyl esters are also given. (*Concluded*)

		Lipids, 1970					
	Commercial oil, 1968,	Triglyceride		Total	Phospholipid		North Sea sand launce
Fatty acid	June	April	July	July	April	July	oil, 1966[a]
18:3ω3	0.41	0.86	0.46	0.55	0.64	0.70	1.0
18:4ω3	2.87	2.62	1.97	2.31	0.54	0.59	2.4
18:4ω1	0.13	0.39	0.25	0.21	0.08	0.03	–
20:2ω6	0.28	0.18	0.16	0.08	0.08	0.09	0.1
?	–	–	–	–	–	0.72	–
20:3ω6	0.26	0.04	0.07	0.06	–	0.03	–
20:3ω3	–	0.05	0.05	–	0.05	trace	0.1
20:4ω6	0.14	0.33	0.24	0.10	0.56	0.46	0.4
20:4ω3	0.29	0.42	0.31	0.20	0.31	0.28	0.6
20:5ω3	10.11	13.28	9.12	11.41	12.29	12.33	10.8
21:5 ?	0.58	0.72	0.29	0.32	0.21	0.22	0.6
22:5ω6	trace	0.07	0.04	0.06	0.41	trace	trace
22:5ω3	1.30	0.58	0.86	0.54	0.95	0.45	1.2
22:6ω3	6.81	7.03	5.12	5.96	26.80	30.94	8.2
Total polyunsaturated acids	27.16	31.05	23.14	26.35	45.91	49.13	27.7
Calculated IV	140.9	150.6	134.4	141.5	204.2	216.9	
Wijs IV	143.0	152.8	142.4	146.9			

[a]From Hølmer 1967.

[b]TMTD = trimethyltridecanoate; TMPD = tetra methylpentadecanoate; TMHD = tetramethylhexadecanoate.

The apparatus was a Perkin–Elmer 900 with the oven at 170 C and with helium at 40 psig as the carrier gas. Quantitation was verified by comparison of total ester components with corresponding chain lengths in hydrogenated samples (Ackman et al. 1967b). Two decimal places are employed in Table 1 solely to show the relative proportions of minor components and there is no implication of this order of accuracy.

Industrial potential of Nova Scotia sand launce oil — The recovery of lipid from the fish taken April 29, 1970, was 6.3%, and from the fish taken July 7, 1970, was 8.1%. On gel chromatography of the lipids the respective recoveries of triglyceride were 82.4% and 77.6%, and of phospholipids 10.2% and 8.6%. The Wijs IV for the oil cooked from the July fish was 148.8, close to the values of 142.4 and 146.9 for the July triglyceride and total lipid. The two triglycerides isolated by chromatography have therefore been considered as comparable to commercial oils from the point of view of fatty acid composition. The experimental IV were low (142.0–152.8) compared to values for oils from North Sea fish (158–178) reported by Hølmer (1967). The oils of herring from southwestern Nova Scotia also show slightly lower IV (105–135) than oils produced in recent years from the larger herring reduced in Denmark (IV about 135–156, Hølmer 1967). This regional difference for both species is suggestive of some comparable dietary or environmental influence (cf. Ackman and Eaton 1970). There are commercial implications since hydrogenation is a relatively expensive process in the conversion of fish oils to edible fat products and low IV oils may be preferred if otherwise suitable.

The physical properties of hydrogenated marine oils are influenced by chain length composition, and sand launce oil is not very different from herring oils (Table 2, see also Ackman and Eaton 1966; Ackman et al. 1967a). The only other small species that we have examined as a potential source of commercial fish body oil, the capelin *Mallotus villosus*, is of comparative interest in that in the triglycerides the percentage of C_{20} is equal to or somewhat less than C_{16}, and C_{18} is about half of these figures, C_{22} is usually less than C_{20} (Ackman et al. 1969). Hydrogenated eastern Atlantic oils from both sand launce and capelin probably differ from herring oils primarily in the comparable $C_{20} \geq C_{22}$ ratio and high proportion of C_{16}.

The unsaponifiable matter in North Sea launce oils has been described by Hølmer (1967). Mostly sterol, presumably cholesterol, it also includes small proportions of hydrocarbons and glyceryl ethers. The North Sea samples averaged 2.49% unsaponifiable materials (range 1.34–3.56%), comparable to our single value of 2.4%. The North Sea work on other species also showed 1.93% average for small (10–12 cm) herring as against 1.58% for larger (15–25 cm) herring. Aside from this level of unsaponifiable material, possibly higher than in herring oils, sand launce oils from Nova Scotia fish appear as suitable for the manufacture of edible fats in Canada as the oils from Danish production that are used for this purpose in Europe.

Biochemical implications of fatty acid compositions — Biochemically, the absence of a large excess of 22:1[1] over 20:1 differentiates triglycerides of sand launce from herring oils (reviewed by Ackman and Eaton 1970, MS 1970). However, the monoethylenic isomer ratios in all chain lengths are quite similar to those in herring oil (Ackman and Castell 1966), Newfoundland turbot (Ackman et al. 1967a), or cod liver oil (Ackman et al. 1967b), suggesting a common mode of biological formation or deposition from food (for Crustacea cf. Ackman et al. 1970; Ackman and Hooper 1970).

We also note that the total 16:1 + 18:1 in our samples remains virtually constant at 21.0, 21.8, and 23.7% in the oil and two triglyceride fractions. Hølmer (1967) found the averages for these acids to be 12.3% and 8.8%, respectively (a total of 21.1%). This uniformity for the total of 16:1 and 18:1 acids across the Atlantic Ocean, and the high proportion in our samples of 18:1ω7 (derived from 16:1ω7) in relation to 18:1ω9, suggest that 16:1 and 18:1 are interchangeable or equivalent as discussed earlier for herring oils (Ackman and Eaton 1966). Hølmer considered the proportion of 16:1 in relation to 18:1 in sand launce to be higher in most oils than in comparable oils from North Sea herring. In the analytical data from our three western Atlantic samples (oil and two triglycerides) total 16:1 is also markedly higher than total 18:1. We have found that 18:1 usually exceeds 16:1 in Canadian Atlantic herring oils (Ackman and Eaton 1966), and agree with Hølmer in these particular relations differentiating between sand launce and herring oils. The important 16:0 is lower in our samples (Table 1) than in the data (average 20.8%) listed by Hølmer, giving a slightly lower total C_{16} (Table 2).

The polyunsaturated fatty acids in the oil and triglycerides showed a high proportion of 18:4ω3, and of 16:4ω1 (together with a small amount of tentatively identified successor acid 18:4ω1). These two acids are associated with phytoplankters (Ackman et al. 1968), and suggest that sand launce, if they do not directly in part feed on phytoplankters (cf. Leim and Scott 1966), at least feed heavily on primary herbivores. Hølmer (1967) also found 18:4ω3 to be in substantial (average 3.5%) proportion. The type of food of Nova Scotia sand launce is not known precisely, but a search of a few euphausiid and copepod lipids for 16:4ω1 turned up only modest levels (Ackman and Hooper 1970; Ackman et al. 1970). Possibly these ω1 acids accumulate in sand launce lipids because they are metabolically inert. Although this opinion is based on the failure of 16:4ω1 to chain-extend in rats (Klenk et al. 1961), there is also a striking absence of 16:4ω1 (and of the associated 16:3ω4) from the physiologically important phospholipids in the sand launce (Table 1). The acids 18:2ω6, 18:3ω6, and 18:3ω3, also of obligatory plant origin, can pass along normal animal biochemical pathways to C_{20} and C_{22} successor acids and need not accumulate.

The phospholipids were comparable in fatty acid composition to phospholipids of herring

TABLE 2. Chain length composition of various sand launce oils and triglycerides compared with an average for eastern Atlantic herring oils.

	Sand launce			Herring	
	Commercial oil, N.S., 1968[a]	Triglyceride, N.S., April, 1970[a]	Triglyceride, N.S., July, 1970[a]	Avg Danish oil[b]	Avg eastern Atlantic oil
C_{14}	8.0	7.3	7.7	7.8	6.7
C_{15}	0.7	0.5	0.7	0.9	0.4
C_{16}	26.9	28.5	31.1	33.1	22.8
C_{17}	0.5	0.5	0.6	1.0	0.4
C_{18}	13.4	16.5	12.6	17.8	17.2
C_{19}	0.1	0.1	0.3	–	–
C_{20}	23.4	24.2	23.2	19.2	23.2
C_{21}	0.5	0.7	0.8	–	0.4
C_{22}	23.1	19.2	20.4	19.3	27.4
C_{24}	1.3	0.8	0.5	0.8	1.4

[a] From hydrogenated esters.
[b] Hølmer 1967.

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[1] Shorthand notation for chain length and number of double bonds. The addition of "ω" gives the position of the double bond closest to the terminal methyl group.

(Addison et al. 1969; Drozdowski and Ackman 1969) and capelin (Ackman et al. 1969).

Comparative data for the "fat" of Japanese *A. personatus* (Ueda and Nagaok 1968) show, in duplicate analyses on one sample, slightly lower (4.9–5.1%) levels of 14:0 and higher levels of 16:0 (16.4–17.1%) than for our samples. The proportions of 16:1 (6.3–6.4%) and 18:1 (9.0–9.4%) are strikingly different from our results (see discussion above), as are the proportions of 20:5ω3 (14.5–14.8%) to 22:6ω3 (25.0–25.7%). In this study there were samples from two lots of fish taken off Hikari City, Yamaguchi Prefecture, in June and August 1966, with quite similar fat properties. The fats were extracted by boiling, which would give mostly triglyceride and include the free fatty acids liberated hydrolytically from phospholipids (or previously liberated by enzyme activity; see Ackman et al. 1969; Addison et al. 1969). These fish are reported to be fat from April to June, and possibly they would be lean (2–3% total lipid) in the period when caught. The phospholipid fatty acids could then alter the fatty acid characteristics of this oil or fat to give the differences from our data cited above. There does not therefore appear to be any evidence for a major biochemical distinction for lipids in *A. personatus* except a low level of fat in the particular samples analysed.

Seasonal distribution data are limited, but the samples used for this study were not obviously ripe and the sand launce may be a late winter spawner (Scott 1968). The depot fat (oil or triglyceride) compositions are thus probably as close to species-typical values as can be obtained. Most of the samples studied by Hølmer (1967) came from corresponding midsummer periods, and showed very substantial variations in major oil components (e.g., 16:0 max 25.1, min 17.0%; 22:1 max 17.0, min 4.6%; 22:6 max 10.4, min 5.4%). A detailed analysis carried out by Hølmer on one sample (EF 22), recalculated for this paper and included in Table 1 for comparative purposes, confirms the proportions of major and minor fatty acids as typical of this species.

Acknowledgments — We wish to thank the Nova Scotia Department of Fisheries for its assistance in this project. The cooperation of Dr G. Hølmer has provided valuable comparative data.

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Some Blood Chemistry Values for the Juvenile Coho Salmon (*Oncorhynchus kisutch*)

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WEDEMEYER, G., AND K. CHATTERTON. 1971. Some blood chemistry values for the juvenile coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Bd. Canada 28: 606–608.

Overlapping Gaussian distribution curves were resolved into normal ranges for 1800 clinical test values obtained from caudal arterial blood or plasma of more than 1000 juvenile coho salmon (*Oncorhynchus kisutch*) held under defined conditions of diet and temperature. Estimated normal blood chemistry ranges were bicarbonate, 9.5–12.6 mEq/liter; blood urea nitrogen (BUN), 0.9–3.4 mg/100 ml; chloride, 122–136 mEq/liter; cholesterol, 88–262 mg/100 ml; $p\mathrm{CO}_2$, 2.6–6.1 mm Hg (10 C); glucose, 41–135 mg/100 ml; hematocrit, 32.5–52.5%; hemoglobin, 6.5–9.9 g/100 ml; total protein, 1.4–4.3 g/100 ml; blood *p*H (10 C), 7.51–7.83. The calculated range of normal acid–base balance vs. water temperature is also presented.

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NORMAL ranges have been tabulated for numerous blood constituents in many of the higher vertebrates and are useful diagnostic tools in human and veterinary physiology and medicine. In contrast, information about the blood chemistry of fishes has been quite fragmentary, being especially limited in terms of expected normal ranges (Wedemeyer and Chatterton 1970). Additional information on normal ranges should advance biomedical studies of fish diseases as veterinary and human medicine have been advanced: through improved diagnoses and knowledge of organ function (Phillips 1958).

Defining a "normal" fish or fish population is difficult. Also, any clinical attempt to prove that such a group is entirely normal would probably be much less sensitive and reliable than the laboratory tests under study. Consequently, a population of clinically healthy fish held under defined conditions must be tested and a statistical method be used to establish normal ranges. The term "normal range" by standard usage is the 95% confidence limit of the mean rather than merely the range of the data. It is set conservatively wide to avoid too many false positive results. In a previous report of blood chemistry values for the rainbow trout (*Salmo gairdneri*), the normal range was calculated by the graphic technique of Hoffman (Wedemeyer and Chatterton 1970). This indirect method gives a reliable estimate of the normal range if any abnormal subpopulations present are small compared with the total sample size. However, it may give too broad an estimate of the actual normal range if any of these subpopulations are of appreciable magnitude.

Recently, a more efficient procedure for resolving overlapping Gaussian distributions has been adapted to clinical chemistry measurements and gives a reliable estimate of the normal range even in the presence of several subpopulations (Bhattacharya 1967; Gindler 1970). With this procedure, data on the blood chemistry of juvenile coho salmon (*Oncorhynchus kisutch*) held under defined conditions were evaluated to estimate some normal clinical chemistry values. The calculated normal ranges for 10 components of blood and plasma

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were determined from 1800 clinical tests on more than 1000 individual blood samples.

Materials and methods — About a month before use, 10- to 15-g juvenile coho salmon were obtained from the Quilcene National Fish Hatchery and held in the laboratory at 10 C in water of 20 ppm hardness ($CaCO_3$); they were maintained on the Abernathy dry diet (Fowler and Burrows 1970), and fasted overnight before sampling (Phillips 1958). Blood was collected directly from the caudal artery into heparinized capillary tubes. Benzocaine anesthesia was used under conditions shown to have no effect on blood chemistry (Wedemeyer 1970). Fresh plasma was used for bicarbonate and CO_2 measurements. Whole blood was tested immediately for hematocrit, hemoglobin, and *p*H. Individual plasma aliquots were stored at -10 C until assayed for blood urea nitrogen (BUN), Cl^-, cholesterol, glucose, and total protein.

Bicarbonate was determined titrimetrically. Hematocrit values were obtained by the standard technique of refrigerated centrifugation to constant cell volume, blood *p*H by electrometric *p*H meter at 37 C and then calculated to 10 C, hemoglobin by photohemoglobinometer, and blood CO_2 by Natelson microgasometer. All other values were determined by previously described methods (Wedemeyer and Chatterton 1970).

Results and discussion — The estimated normal clinical chemistry ranges for bicarbonate, BUN, chloride, cholesterol, glucose, total protein, hematocrit, hemoglobin, arterial CO_2, and *p*H are shown in Table 1. Five corresponding values for juvenile rainbow trout held under similar conditions are listed for comparison. The hematocrit and hemoglobin ranges given here, though relatively wide, are in good agreement with mean values reported for fingerling chinook salmon (*Oncorhynchus tshawytscha*) held at similar temperatures (Thomas et al. 1969). The blood *p*H values with the correction factor of Rosenthal (1948) were used to derive an acid-base balance chart (Fig. 1) that relates the estimated normal *p*H range of juvenile

TABLE 1. Estimated normal ranges for the blood chemistry of juvenile coho salmon. Corresponding ranges for juvenile rainbow trout held under similar conditions are given for comparison (Wedemeyer and Chatterton 1970).

Chemical component (caudal artery)	Coho salmon	Rainbow trout
Bicarbonate (*mEq/liter*)	9.5–12.6	–
BUN (*mg/100 ml*)	0.9–3.4	0.9–4.5
Chloride (*mEq/liter*)	122–136	84–132
Cholesterol (*mg/100 ml*)	88–262	161–365
pCO_2 (*mm Hg (10 C)*)	2.6–6.1	–
CO_2 (*ml/100 ml*)	24.2–27.8	–
Glucose (*mg/100 ml*)	41–135	41–151
Hematocrit (%)	32.5–52.5	–
Hemoglobin (*g/100 ml*)	6.5–9.9	–
*p*H (*10 C*)	7.51–7.83	–
Total protein (*g/100 ml*)	1.4–4.3	2.0–6.0

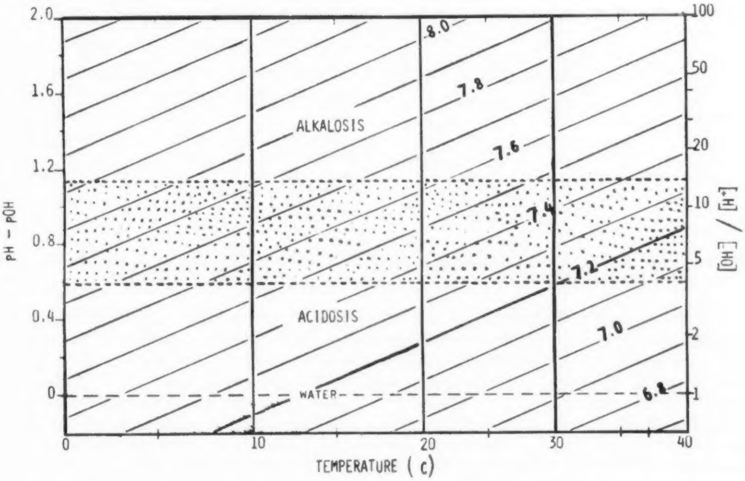

FIG. 1. Calculated range of normal acid-base balance in the juvenile coho salmon as a function of water temperature. Stippled band is the estimated range in $(OH^-)/(H^+)$ ratio; line labeled "water" indicates the *p*H of neutral water at the given temperatures.

coho salmon to environmental temperature (Howell et al. 1970). The temperature scale is calculated as $1/k^2$ but is re-expressed in degrees C and thus is not linear. In fishes, blood *p*H alone does not reliably reflect the acid–base status. Insufficient attention is usually given to the fact that *p*H + *p*OH = 10^{-14} only at 24 C. The expression of blood *p*H as a function of *p*H – *p*OH or its antilog, the $[OH^-]/[H^+]$ ratio, allows an estimation of the range of acidosis and alkalosis at any environmental temperature. Thus a juvenile coho salmon with a blood *p*H of 7.5 might be normal or acidotic, depending on the water temperature.

Estimates of normal clinical chemistry ranges for this species should considered a guide to expected values in populations of like age-groups under corresponding environmental conditions. For example, levels for total protein or BUN outside these ranges in similar circumstances, may indicate dietary effects rather than pathological processes. Conversely, substances involved with vital functions, e.g., blood chloride and *p*H, are less subject to dietary changes. Barnhart (1969) has documented some of these effects for the rainbow trout.

Defining normal clinical chemistry ranges applicable to a wider range of rearing conditions, and to additional blood constituents, should help to optimize environmental conditions and further the use of fish as experimental animals in biomedical and toxicological research. However, emphasis must be placed on the use of normal ranges, since mean values alone will be of limited clinical use.

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A Hermaphroditic Specimen of Weathervane Scallop, *Patinopecten caurinus*, in Alaska

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HENNICK, D. 1971. A hermaphroditic specimen of weathervane scallop, *Patinopecten caurinus*, in Alaska. J. Fish. Res. Bd. Canada 28: 608–609.

Hermaphroditism in sea scallops is a relatively rare phenomenon. Observations on two hermaphroditic specimens of *Placopecten magellanicus* have been documented in the literature. Recent studies on the weathervane scallop, *Patinopecten caurinus*, in Alaska, have resulted in the discovery of a single hermaphroditic individual from sexual examination through dissection of more than 25,000 individuals over a 3-year study period. The specimen appeared to be a functional hermaphrodite, as both male and female follicules containing well-developed eggs and sperms were clearly evident from microscopic examination. Shell examination showed the animal had formed seven annual rings indicating the animal had spawned more than one season, since weathervane scallops mature after formation of the third annual ring.

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VARIOUS investigators have well documented that hermaphroditism of normally dioecious mollusks is not an uncommon phenomenon (Coe 1942, 1945; Merrill and Burch 1960). The condition, however, is rare among sea scallops. Two hermaphroditic specimens of *Placopecten magellanicus* were collected on the east coast of the United States (Merrill and Burch 1960), and other investigators have reportedly observed them on occasion.

In 1968 a commercial fishery for weathervane scallops, *Patinopecten caurinus*, developed in Alaska. This fishery spurred interest and initial studies on the species. Relatively little is known or reported in the literature on this species, and nothing on hermaphroditism, hence this note is of some interest.

Scallops in Alaska reach sexual maturity after formation of the third annual ring, and major spawning takes place during early July. Sexes are separate and mature individuals are easily identified by the characteristic bright orange of the ovary and white of the testis (Hennick 1970). Dissection studies, both in the field and in the laboratory, under direction of the author during 1968 through August 1970 on more than 25,000 commercially caught scallops, failed to reveal an incidence of hermaphroditism. However, recent dissection of an additional 100 live scallops in the laboratory resulted in discovery of a single hermaphrodite. Externally the animal appeared normal and seven annual rings had formed on the upper valve, an indication that the animal had reached sexual maturity. Microscopic examination revealed the gonad contained about equal portions of orange ovarian and white testicular tissues. Apparently the animal was a functional hermaphrodite as both male and female portions of the gonad appeared to be in the early recovery stage after the early summer spawning season. Also, it is likely spawning had occurred over more than one season, since scallops reach maturity after formation of the third annual ring, although only one annual spawning occurs (Hennick 1970). The proximal part of the gonad formed the ovary with the distal portion forming the testis, similar to hermaphroditic individuals of *Placopecten magellanicus* (Merrill and Burch 1960). Histological examination clearly showed that both well-defined eggs and sperm were being formed within the follicles of the respective portions of the gonad. The yolk and nucleus of developed eggs were clearly evident as were the head and long flagella of the developed sperm cells. In the region where testicular and ovarian tissues joined, there appeared to be an interspersing of both male and female follicles; no ambisexual follicles were observed. Not all follicles were fully developed at the time observations were made; however, a sufficient portion were developed to establish that this individual was a hermaphrodite, a fact that has not previously been reported in the literature.

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Concentrations of Dieldrin in the Blood and Brain of the Green Sunfish, *Lepomis cyanellus*, at Death

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HOGAN, R. L., AND E. W. ROELOFS. 1971. Concentrations of dieldrin in the blood and brain of the green sunfish, *Lepomis cyanellus*, at death. J. Fish. Res. Bd. Canada 28: 610–612.

When green sunfish, *Lepomis cyanellus*, were exposed to concentrations of dieldrin averaging 6 ppb for periods ranging from 124 to 139 hr, the concentrations of dieldrin in the blood and brain at death were approximately 6.0 and 9.0 μg/g. Surviving fish had concentrations markedly lower than those that died. A correlation coefficient of 0.95 was found between concentrations of dieldrin in the blood and in the brain, water dieldrin concentrations having a slight influence on the concentrations in the blood at death. Surviving fish exhibiting severe poisoning symptoms had higher concentrations of dieldrin in the blood and brain than did those showing moderate to minimal symptoms.

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THERE have been several studies of concentrations of various pesticides and their distributions within the organs of fish, but a limited number have been of a toxicological nature. Mount et al. (1966) established the long-term lethal concentration of endrin in the blood of the channel catfish, *Ictalurus punctatus*, and suggested the use of blood as an indicator tissue for endrin caused fish-kills. Lethal concentrations of endrin in the blood were determined for the gizzard shad, *Dorosoma cepedianum*, by Brungs and Mount (1967).

This study was conducted to determine the concentrations of dieldrin in the blood and brain of the green sunfish, *Lepomis cyanellus*, at death, and the relation between blood and brain dieldrin concentrations in the blood and brain.

Methods — Green sunfish were obtained from a pond in the Kellogg Bird Sanctuary near Hickory Corners, Michigan, and were held a minimum of 3 weeks in a 500-gal holding tank with a carbon-filtered water recycling system. Blood and brain samples taken from six fish to determine the presence of dieldrin (1,2,3,4,10,10,hexachloro-exo-6,7-epoxy-1,5,5a,5,6,7,8,8a octahydro-1,4-endro,exo-5,6-dimentha-nonaphthalene) were negative.

After the holding period, the fish were placed in a 70-liter test tank containing carbon-filtered water maintained at 18 C. An attached pump and glass wool filter facilitated complete mixing of the water and dieldrin and eliminated bacteria and detritus buildup. Dieldrin was added to the tank by a marriotte bottle containing 4 liters of water and 2 mg (500 ppb) of dieldrin. The pesticide had been added to the marriotte bottle with 5 ml of acetone. The bottle was calibrated to add from 2 to 10 ml/min to the test tank. Rates of addition throughout each of the three replications of 10 fish each ranged from 4.0 to 6.5 ml/min.

Three tests were carried out to determine the dieldrin losses in the test tank and filter attributable to glass absorption. In each test 1500 μg of dieldrin were added to the covered, nonaerated tank, and six water samples were taken at intervals between 1 and 24 hr. An average of 55% of the initial dieldrin dosage was adsorbed in 24 hr, 45% being adsorbed within the first 8 hr (Fig. 1). Decreases in the concentration of dieldrin in the water from the 3rd to the 24th hr averaged 1.19%/hr. Aeration losses, as discussed by Mount (1962) and Bowman et al. (1964), were computed after the 24th hr in one of the absorption tests. The water was vigorously aerated for an additional 24 hr after which a water sample was taken to determine the dieldrin concentration. Allowing for the 1.19%/hr adsorption loss determined earlier, the 24-hr aeration loss was found to be 10.44%.

Twenty-four hours before the initiation of each replication, 1 mg of dieldrin was added to the test tank to allow for glass adsorption losses. Water samples were taken at the end of the 24-hr period to determine concentrations of dieldrin existing just prior to the introduction of the fish. Three replications with a mean dieldrin concentration of 6 ppb (Fig. 2) were run, the duration of each being determined by the length of time required for 50% of the test fish to die. The criteria for death were set at that point where there was no longer opercular action and the fish could not be stirred to movement by its removal from the tank.

All fish that died were removed immediately from the tank and fish that survived were killed using a 10%

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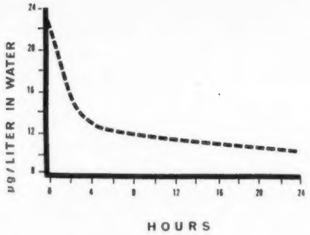

FIG. 1. Adsorptive losses of dieldrin in test tank and filter.

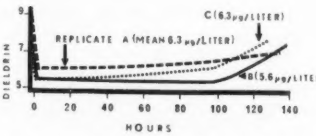

FIG. 2. Concentrations of dieldrin (μg/liter) in test tank water as determined from an average of 25 samples of 100 ml of water per replicate.

tricaine methanesulphonate solution. Blood samples taken by severing the caudal peduncle were weighed and frozen. To facilitate the removal of the brains, the brain cases were removed and fixed for 24 hr in a 10% formalin solution, after which the brains were excised and weighed. Length, weight, sex, and, in the case of surviving specimens, symptoms of poisoning were recorded.

Blood and brain samples were assayed using a modification of the method described by Schafer et al. (1963). The samples were saponified with alcoholic sodium hydroxide over a hot plate to allow a slight refluxing action. After cooling, the samples were extracted into petroleum ether and concentrated to 15 ml. Measured volumes were then injected into an Aerograph 550B electron capture gas chromatograph equipped with a column of Chromosorb W packing coated with a 4% QF-1 liquid phase. Operating temperature was 175 C and the nitrogen flow 35 ml/min. Three aliquots of each sample were injected and the resultant peaks compared to 0.1 μg/ml standards, which were run after each sample injection. Standards were prepared with Shell analytical standard 99+% HEOD (dieldrin). Reagent blanks and blood and brain samples from the control fish were consistently negative. Five samples each of blood and brain were fortified with dieldrin and interspersed with the normal samples in order to determine efficiency of extraction. The average extraction efficiency was 94.92 ± 8.8% for the blood, and 92.8 ± 9.7% for the brain.

Results and discussion—All but three of the fish that died had blood dieldrin concentrations above 5.17 μg/g, and all but one of those that survived had concentrations less than this. The concentrations for the two groups (Table 1) were different at $P = 0.001$ (Mann-Whitney U test). The variance and standard deviation for the blood dieldrin concentrations of fish that died were 1.180 and ±1.086, respectively, the concentration of dieldrin in the water at time of death appearing to influence blood dieldrin concentration (Fig. 3). Regression analysis of the blood and water dieldrin concentrations was significant at $P = 0.01$ with 13 degrees of freedom.

As was the case with the blood, higher concentrations of dieldrin were found in the brains of fish that died than in those of survivors. All but one of the fish that died had brain dieldrin concentrations above 8.70 μg/g, and all but one of those that survived had concentrations below this figure. The Mann-Whitney U test indicated differences between the two sample groups to be significant at $P = 0.001$. Values for the variance and standard deviation of the dieldrin concentrations in the brains of the dead fish were 4.049 and ±2.012,

TABLE 1. Mean concentrations (μ/g) of dieldrin in blood and brain of green sunfish in relation to observed symptoms, physical characteristics, and duration of exposure. Of the fish tested, 14 died, 9 were hyperactive, and 7 had minimal symptoms.

	Died	Hyperactive	Symptoms minimal
Concn in blood	5.65(1.18)[a]	4.84(2.18)	3.94(0.70)
Concn in brain	10.31(4.05)	7.67(1.21)	5.08(1.32)
Mean length of fish (*mm*)	135(112.50)	131(41.80)	139(11.00)
Mean wt of fish (*g*)	48(109.50)	42(37.00)	53(13.00)
Mean duration of exposure (*hr*)	111(375.00)	133(27.00)	132(45.00)

[a] Variance.

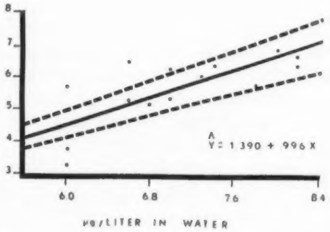

FIG. 3. Regression of dieldrin concentration in the blood of green sunfish on that in the water at time of death. Broken lines, 95% confidence limits.

respectively. A correlation between dieldrin concentrations in the blood and brain was indicated by a correlation coefficient of 0.95 with 29 degrees of freedom and regression analysis was significant at $P = 0.05$.

Symptoms of poisoning observed during the study were similar to those described by Ferguson et al. (1964) for the chlorinated hydrocarbon pesticides. The first noticeable symptom was a rising toward the surface of the water. A general darkening of body coloration accompanied by formation of dark vertical bars occurred, followed by a period of hyperactivity characterized by rapid pectoral and opercular action, erratic swimming or darting, a gradual loss of equilibrium, and death. Of the 16 fish surviving an average exposure time of 132 hr, 9 exhibited hyperactivity and 7 showed only a general darkening of coloration or no symptoms (Table 1). Differences in blood dieldrin concentrations between these two surviving groups were significant at $P = 0.025$, and differences in brain dieldrin concentrations at $P = 0.001$.

The data indicate the concentrations of dieldrin in the blood of the green sunfish at death to be approximately 6.0 µg/g. This concentration appears high, but is within expected limits on the basis of values for endrin obtained in studies of long duration. Mount et al. (1966) in a 44-day study established lethal blood endrin concentrations for the channel catfish of 0.28 µg/g and above, and Brungs and Mount (1967) in an 8-day study found the lethal concentrations for the gizzard shad to be 0.10 µg/g. Henderson et al. (1959) and Katz (1961) studying 96-hr TL_m values for dieldrin and endrin, found endrin toxicity to be 10–20 times as great as that of dieldrin.

The concentration of dieldrin in the water appeared to exert a slight influence on the concentration in the blood at death. This slight effect might be expected in short-term studies, which make use of relatively high concentrations of dieldrin in the water.

The data on brain dieldrin concentrations indicate the concentration at death to be approximately 9.0 µg/g. The concentrations in the brain were more variable than those in the blood; however, a clear distinction was evident in dieldrin concentrations between fish that died and those that survived. Concentrations of dieldrin in the brain correlated with those in the blood ($r = 0.95$ with 29 degrees of freedom) and dieldrin concentrations in both blood and brain showed a relation to categories of observed symptoms, including death, in the test fish.

Some investigators have presented data that indicate a limitation that must be considered in use of blood and brain concentrations of chlorinated hydrocarbons as a diagnostic tool in determining causes of fish-kills. Ferguson et al. (1964), working with endrin and dieldrin, found a difference in 36-hr TL_m limits between fish that had developed a resistance to these compounds through extended previous exposure and those that had not developed a resistance. Ludke et al. (1968), studying endrin and its effects on golden shiners, *Notemigonus crysoleucas*, with a developed resistance, found whole body concentrations of endrin 82 times and blood concentrations 64 times as great as those found in nonresistant fish killed by exposure.

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Some Enzymatic Properties of Plasma Esterases from Channel Catfish (*Ictalurus punctatus*)

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HOGAN, J. W. 1971. Some enzymatic properties of plasma esterases from channel catfish (*Ictalurus punctatus*). J. Fish. Res. Bd. Canada 28: 613–616.

Rates of ester hydrolysis by plasma enzymes from five fish species ranged from 12.8 to 46.6 μmoles acetylcholine hydrolyzed/ml plasma per hr. Enzymes in channel catfish (*Ictalurus punctatus*) plasma hydrolyzed the substrates acetylcholine, phenyl acetate, and glyceryl triacetate at rates of 28.1, 96.7, and 6.8 μmoles substrate hydrolyzed/ml plasma per hr, respectively. Esterolytic activity was attributed, in part, to an acetylcholinesterase-like enzyme. In vitro organophosphate inhibition of enzymes in channel catfish plasma was similar to that previously reported for catfish brain acetylcholinesterase (EC 3.1.1.7, acetylcholine acetyl-hydrolase).

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THE current controversy over organochlorine pesticides may well lead to increased usage of the highly toxic, but less persistent, organophosphorus compounds. Though these may be ephemeral in aquatic ecosystems, their biological effects could be long-lasting and development of methods useful in assessing those effects is desirable.

Despite certain drawbacks, procedures based on the esterase activity present in whole or fractions of human blood have been routinely used for a number of years to assess the extent of exposure to esterase-inhibiting compounds (Gage 1967). With this in mind, I investigated fish blood to determine if there were enzymes present that could be used to assess some of the effects of organophosphorus pesticides in fish populations.

A preliminary survey of five fish species revealed that measurable esterolytic activity was present in plasma. The rates of acetylcholine (ACh) hydrolysis for the species tested were grass pickerel (*Esox americanus vermiculatus*), 46.6 ± 4.3 μmoles ACh hydrolyzed/ml plasma per hr; black bullhead (*Ictalurus melas*), 28.2 ± 5.5; channel catfish (*Ictalurus punctatus*), 28.1 ± 6.6; yellow bullhead (*Ictalurus natalis*), 17.7 ± 4.9; and northern pike (*Esox lucius*), 12.8 ± 7.7. Of this group, channel catfish appeared to be the best species for further study because of their relatively high plasma esterase activity, ready availability, and relative ease of feeding and holding. This paper, therefore, reports the characterization of the esterase activity in channel catfish plasma.

Materials and methods — Channel catfish were obtained from the Fish Farming Experimental Station, Stuttgart, Arkansas. All other fish were obtained from National Fish Hatcheries located in Corning, Arkansas, and Guttenberg, Iowa.

Fish were anesthetized in 0.2 g/liter (w/v) of ethyl-*m*-aminobenzoate methanesulfonate (M.S. 222) before drawing blood. Student's *t*-test (Fisher 1924) was used to statistically compare mean activity levels in plasma from anesthetized and unanesthetized channel catfish and the results indicated the probability of M.S. 222 having any effect was $P < 0.20$.

The plasma fraction was separated by centrifuging whole blood for 10 min at 1500 rpm in an International Model CL centrifuge using a 221 head. All centrifuge tubes were coated with a 10% heparin solution. Most plasma samples were diluted (v/v, 1/5) with a bicarbonate buffer (0.357 M sodium bicarbonate and 0.164 M sodium chloride, gassed with 95% N_2–5% CO_2 to *p*H 7.6) immediately before analysis. Samples were kept at 5 C before analysis and, with the exception of some storage experiments discussed below, were used immediately after preparation.

The following water-soluble salts of choline esters were used as substrates: acetylcholine iodide (ACh), propionylcholine-*p*-toluenesulfonate (PrCh), butyrylcholine-*p*-toluenesulfonate (BuCh), acetyl-β-methylcholine bromide (MeCh), and benzoylcholine chloride (BzCh). Noncholine esters used as substrates were phenyl acetate (PhA), phenyl-*n*-butyrate (PhB), glyceryl triacetate (TA), glyceryl tri-*n*-butyrate (TB), and methyl-*n*-butyrate (MeB).

The following compounds were tested as esterase inhibitors: eserine sulfate; 1,5-*bis*(4-trimethylammonium phenyl)pentan-3-one diiodide (62C47); di*iso*propyl phosphorofluoridate (DFP); 2,2-dichlorovinyl dimethyl phosphate (dichlorvos); *O,O*-diethyl-*O*-*p*-nitrophenyl phosphorothioate (parathion); diethyl *p*-nitrophenyl phosphate (paraoxon), and diethyl mercaptosuccinate *S*-(*O,O*-dimethylphosphorodithioate) (malathion).

Divalent metal chloride salts tested for their effect on esterase activity included calcium chloride ($CaCl_2$),

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cobalt chloride ($CoCl_2 \cdot 6H_2O$), cupric chloride ($CuCl_2 \cdot 2H_2O$), magnesium chloride ($MgCl_2 \cdot 6H_2O$), manganous chloride ($MnCl_2 \cdot 4H_2O$), and nickel(ous) chloride ($NiCl_2 \cdot 6H_2O$). All divalent cations were tested at a 1 mM concentration. Sodium chloride was omitted from the buffer for these experiments.

The manometric procedure outlined by Knowles and Casida (1966) was used to determine esterase activity, and effect of inhibitors and divalent cations. Esterase activity with the water-soluble salts of the choline esters was determined as a function of substrate concentration. The negative logarithm of the molar substrate concentration is designated by the abbreviation *p*S. Water soluble noncholine esters were used at a concentration of 10 mM. Triton X-100 (Rohm and Haas, Philadelphia, Pennsylvania) (0.25%) was used to emulsify noncholine esters that were not water soluble at the 10 mM level. Esterase activity is expressed as μmoles of substrate hydrolyzed per ml plasma per hr.

Anticholinesterase agents and divalent cations were preincubated with the plasma sample for 40 min before the addition of ACh. Inhibition caused by anticholinesterase agents is denoted as either pI_{50} (negative logarithm of the molar concentration of anticholinesterase agent needed for 50% inhibition of the ACh hydrolyzing enzyme), or percentage inhibition at a given molar concentration of anticholinesterase agent. The effect of divalent cations on the ACh hydrolyzing enzyme is expressed as percent of activity in unexposed control samples.

All data on esterase activity are means from at least duplicate determinations.

Total protein concentration was determined for a limited number of samples. Analyses were conducted with the Folin–Ciocalteau method (1927) as modified by Lowry et al. (1951) and Miller (1959). Fresh bovine serum albumin was used as the standard. Results of 24 analyses indicated a protein concentration of 7.4 ± 0.7(SD) mg/ml in the diluted (v/v, 1/5) plasma samples.

Results — The rate of hydrolysis of ACh by channel catfish plasma was linear for at least 45 min after the reaction was initiated by tipping the flasks (Fig. 1). ACh hydrolysis was proportional to the plasma concentration in the flasks (Fig. 2).

Plasma esterases consistently exhibited bell-shaped activity–substrate concentration curves for ACh, PrCh, and MeCh (Fig. 3). Optimum esterase activity was obtained at a substrate concentration of 10 mM for all three choline esters. Hydrolysis of BuCh and BzCh was very slow and could not be accurately estimated manometrically.

Eserine, with a pI_{50} of 6.6, was the most potent of the seven inhibitors tested. Of the organophosphates tested, malathion was the least potent and paraoxon was the most potent in vitro esterase inhibitor (Table 1).

In a test of the effect divalent cations have on plasma esterase activity, Ca, Ni, and Cu caused 2, 10, and 20% inhibition, respectively. Under similar conditions Mg and Mn caused esterase activity to be increased by 6 and 10%, respectively; Co had no measurable effect.

With the exception of MeB, all of the noncholine esters tested were hydrolyzed by channel catfish plasma. The phenyl esters were hydrolyzed rapidly, with PhA being hydrolyzed 3.4 times as fast as ACh. The enzymes hydrolyzing the aromatic and aliphatic esters were very sensitive to the organophosphorus pesticide dichlorvos, but were less inhibited by eserine (Table 2).

A catfish plasma sample that had been divided into aliquots and frozen and held at −20 C had an activity of 28.9 ± 1.3 μmoles ACh hydrolyzed/ml

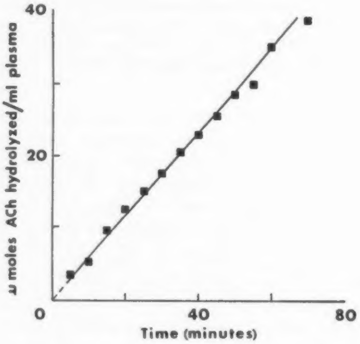

FIG. 1. Hydrolysis of acetylcholine (10 mM) by channel catfish plasma esterases as a function of time.

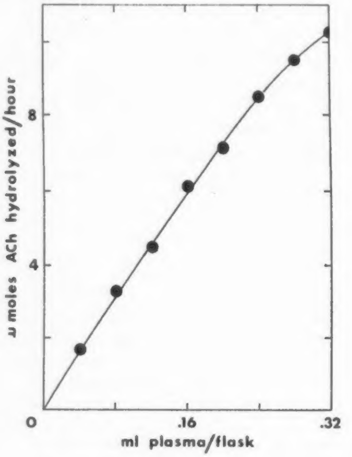

FIG. 2. Hydrolysis of acetylcholine (10 mM) by channel catfish plasma esterases as a function of plasma volume.

plasma per hr for samples analyzed after 5, 14, 21, 28, 90, and 180 days storage. The esterase activity in an aliquot of this sample prior to freezing was 28.9 µmoles ACh hydrolyzed/ml plasma per hr. Although this experiment was limited, I consider that freezing and storage had little effect on the ACh-hydrolyzing enzymes in the plasma.

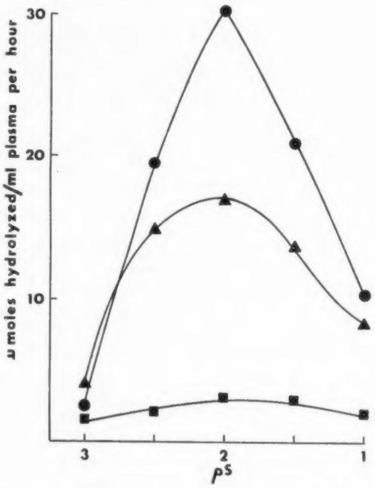

FIG. 3. Hydrolysis of acetylcholine (solid circles), propionylcholine (solid triangles), and acetyl-β-methylcholine (solid squares) as a function of substrate concentration (*p*S).

TABLE 1. Inhibition of esterase activity by several anticholinesterase compounds. Each pI_{50} value is the negative logarithm of the molar concentration of anticholinesterase agent needed to cause 50% inhibition of the ACh-hydrolyzing enzyme.

	Plasma esterase		Brain AChE[a]	
Compound	pI_{50}	Slope[b]	pI_{50}	Slope
Eserine	6.6	0.43	6.7	0.49
Paraoxon	6.4	1.43	6.7	1.25
Dichlorvos	6.2	1.00	6.3	1.25
DFP	5.9	1.25	6.1	0.91
62C47	5.6	0.77	5.4	1.05
Parathion	4.1	0.42	4.2	1.00
Malathion	3.2	0.53	3.5	0.77

[a] Data taken from Hogan and Knowles 1968.
[b] Slope = $1/(pI_{20}-pI_{80})$.

Discussion — The plasma enzymes that hydrolyzed the choline ester substrates exhibited some of the characteristics of an AChE. For instance, the rate of choline ester hydrolysis was greatest with ACh and was inversely proportional to acyl chain length (ACh > PrCh > BuCh). In addition, excess choline ester substrate caused inhibition of activity, as did a relatively low concentration of eserine. Hydrolysis of BuCh and BzCh was insufficient for detection manometrically, but MeCh was hydrolyzed at a detectable rate. All of these distinctions are used by Heath (1961) to differentiate between AChE and cholinesterase (EC 3.1.1.8, acylcholine acyl-hydrolase). On the other hand, the rapid rates of hydrolysis of noncholine ester substrates suggest the presence of at least one or more additional esterolytic enzymes.

The finding that dichlorvos, an organophosphate, caused greater inhibition of noncholine ester hydrolysis than eserine (Table 2) is similar to the results reported by Augustinsson (1959) for five species of teleostean fishes.

In some respects channel catfish plasma esterases are very similar to the esterase present in northern pike plasma (Augustinsson 1959). Specifically, channel catfish and northern pike (Augustinsson 1959) hydrolyzed PhA more rapidly than any other noncholine ester substrate tested. In addition, the relative rates of hydrolysis of choline esters were very much alike. For instance, ACh was hydrolyzed about 10 times as fast as MeCh by both species. Augustinsson (1959) concluded that the main esterase present in pike plasma was an aliesterase (carboxylesterase), and that only a small amount of AChE was present. This may

TABLE 2. Means and standard deviations of esterase activity of channel catfish plasma using choline and noncholine esters as substrates, and percentage inhibitions of esterase activity at a given molar concentration of anticholinesterase agent.

	Activity	% inhibition	
Substrate	(*µmoles substrate hydrolyzed/ml plasma per hr*)	Eserine 10^{-4} M	Dichlorvos 10^{-5} M
Acetylcholine	28.1±6.6	100	94
Glyceryl triacetate	6.8±2.1	77	86
Glyceryl tri-*n*-butyrate	38.0±10.8	18	97
Phenyl acetate	96.7±24.0	43	96
Phenyl butyrate	77.9±16.1	12	93

also be true of channel catfish plasma; however, a larger amount of AChE appears to be present.

Data obtained in this study on the in vitro inhibition of ACh-hydrolyzing enzymes by anticholinesterase agents were compared with data previously reported (Hogan and Knowles 1968) for channel catfish brain AChE. Differences in sensitivity are minor for these enzymes. The ACh-hydrolyzing enzymes in catfish plasma, however, are much less sensitive than brain AChE to the effects of various divalent cations. The greatest difference in effect was noted with Cu; brain AChE was 100% inhibited, but the plasma esterases hydrolyzing ACh were only 20% inhibited.

Paraoxon, the toxic in vivo metabolite of parathion, was a much more potent in vitro inhibitor of both plasma and brain ACh-hydrolyzing enzymes than parathion. The results of Henderson and Pickering (1958) indicate that the in vitro potency of paraoxon and parathion are reflected by their in vivo toxicity. They reported 96 hr TL_m values of 1.6 and 0.25 mg/liter for parathion and paraoxon, respectively, with fathead minnows (*Pimphales promelas*).

This study characterized the esterase activity present in channel catfish plasma. The procedure employed could be used to assess in vivo esterase inhibition caused by organophosphorus pesticides.

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Giant American Brook Lampreys, *Lampetra lamottei*, in the Upper Great Lakes[1]

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MANION, P. J., AND H. A. PURVIS. 1971. Giant American brook lampreys, *Lampetra lamottei*, in the upper Great Lakes. J. Fish. Res. Bd. Canada 28: 616–620.

Five female American brook lampreys, *Lampetra lamottei*, collected in lakes Michigan and Huron averaged nearly twice as long and about six times as heavy as American brook lampreys of normal size. Three factors suggested that the giant lampreys may have fed parasitically after metamorphosis: morphological adaptations of the species for parasitic life, their large size, and absence of extremely large ammocetes among a million sampled.

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THE American brook lamprey, *Lampetra lamottei* (Lesueur), a small, nonparasitic lamprey that inhabits cool streams, is common in tributaries of the upper Great Lakes. Many thousands have been examined during activities related to the experimental control of the sea lamprey, *Petromyzon marinus* Linnaeus, in the Great Lakes; adults and larvae have been collected during chemical treatments (Applegate et al. 1961), from mechanical and electrical weirs (Applegate 1961; McLain et al. 1965), and by electrofishing during stream

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surveys. An estimated 2 million lampreys, including 118,000 adult and larval American brook lampreys, were collected and examined by personnel of the U.S. Fish and Wildlife Service in 1950–70 (Braem and King 1971).

This report presents morphological data on five giant American brook lampreys — all female — captured in 1960 and 1961 in tributaries of Lakes Huron and Michigan.

Materials and methods — Two of the giant lampreys (specimens OR-1 and OR-2, Table 1) were captured in the Ocqueoc River, Presque Isle County, Michigan, on the northwestern shore of Lake Huron. The other three specimens were collected in tributaries of Lake Michigan: one (CR) in the Cedar River, Menominee County, Michigan, near the Michigan–Wisconsin border; and two (CLR-1 and CLR-2) in the Carp Lake River, Emmet County, Michigan, near the Straits of Mackinac. Specimens OR-1, OR-2, and CR were taken in electric weirs while migrating upstream; CLR-1 and CLR-2 were caught in an inclined-plane trap while moving downstream. Although personnel of the U.S. Fish and Wildlife Service in the Great Lakes area were alerted to watch for more of these specimens, none were collected after 1961. The operation of collecting devices was discontinued on the Carp Lake River in 1962 and on the Cedar River in 1966, but the weir on the Ocqueoc River has remained in operation. Structures (weirs, traps, or related electrical fields) at which the collection of giant American brook lampreys could be expected were operated during 1–20 spawning seasons on about 167 tributaries of the upper Great Lakes in 1950–70.

The specimens were preserved in 10% formalin at the time of capture and later transferred to 70% alcohol. Total length was measured to the nearest millimeter and weight to the nearest 0.1 g (after preservation). The identifications were based on characters described by Vladykov (1949), and were verified by R. M. Bailey, Curator of Fishes, University of Michigan Museum of Zoology, Ann Arbor, Mich.

The various characters that were measured (with calipers, to the nearest millimeter on the left side of each of the five lampreys) or counted, and sources of the methods used, were: total length, eye diameter, oral disc diameter, length over gill opening, snout length, body depth, and myomere numbers (Hubbs and Trautman 1937); intestine diameter and dentition (Vladykov 1951); and body length, first dorsal fin height, second dorsal fin height, and caudal fin height (Manion and Stauffer 1970). The diameters of 100 eggs from the midsection of the ovaries of each of the five lampreys were measured to the nearest 0.01 mm with an ocular micrometer.

Size of the giant lampreys — The size of the five giant lampreys was compared with the average size for this species, and with three parasitic species of *Ichthyomyzon* (Table 2) to show that parasitic lampreys are the only species that approach the size of the giant lampreys. The giant lampreys (Fig. 1) were nearly twice the average length and about six times the average weight of 375 adult American brook lampreys from Lakes Superior and Huron, and were 40 mm longer than the average for 124 adult silver lampreys, *I. unicuspis* Hubbs and Trautman, from Lake Superior (average weights of the two species were similar). The giant lampreys were 70 and 97 mm longer than the average reported for *I. castaneus* Girard and *I. bdellium* (Jordan) by Hubbs and Trautman (1937).

Morphological characters — Most of the measurements of the various characters for each of the five specimens (Table 3), expressed in terms of percentage of total length, were within the expected range for American brook lampreys. The average myomere count (72; range 70–73), however, exceeded that for American brook lampreys (68) as determined by Vladykov (1949). Although the oral disc and teeth of the giant lampreys were

TABLE 1. Data for five giant American brook lampreys collected in the upper Great Lakes.

Specimen	Stream	Date	Gear	Length (*mm*)	Wt (*g*)	Disposition[a]
OR-1	Ocqueoc R.	May 24, 1960	Electric weir	260	37.2	UMMZ 177337
OR-2	Ocqueoc R.	June 7, 1961	"	295	37.6	HBBS
CR	Cedar R.	May 22, 1961	"	299	46.0	MSLC
CLR-1	Carp Lake R.	May 17, 1961	Inclined plane trap	286	41.3	UMMZ 178943
CLR-2	Carp Lake R.	May 23, 1961	Inclined plane trap	290	44.7	UMMZ 178944
Avg	–	–	–	286	41.4	–

[a] UMMZ = University of Michigan Museum of Zoology; HBBS = Hammond Bay (Michigan) Biological Station, and MSLC = Marquette (Michigan) Sea Lamprey Control, both of the U.S. Fish and Wildlife Service.

considerably larger than in normal brook lampreys, the dentition and cusp counts were about the same as those described by Vladykov (1949). The numbers of rows of supraoral lamina and maxillary, mandibular, and lateral labials were the same as those described by Vladykov (2, 3, 1, and 0, respectively). Infraoral lamina averaged 7 (range 6–8), enlarged laterals 5 (range 3–6), and marginals (rows) 1 (range 0–1) compared with averages of 8, 6, and 1, respectively, for lampreys from Quebec. The coloration of the body was the same as that described by Vladykov for American brook lampreys.

TABLE 2. Comparison of the size of five giant American brook lampreys with that of average specimens of American brook lampreys and three parasitic species of *Ichthyomyzon*. Ranges in average length and weight in parentheses.

Species and data source	Avg length (*mm*)	Avg wt (*g*)
	Lampetra lamottei	
Average specimens		
Gage (1893)	156(143–170)	–
Vladykov (1949)	(112–187)	–
Vladykov (1951)	143(116–158)	5.2(3.0–7.7)
Present study	154(111–196)	7.2(3.6–15.0)
Giant specimens		
Present study	286(260–299)	41.4(37.2–46.0)
	Ichthyomyzon unicuspis	
Hubbs and Trautman (1937)	224(103–328)	–
Vladykov (1951)	255(201–312)	45.1(13.5–75.0)
Present study	246(201–305)	39.2(26.0–73.0)
	Ichthyomyzon castaneus	
Hubbs and Trautman (1937)	216(105–310)	–
	Ichthyomyzon bdellium	
Hubbs and Trautman (1937)	189(124–259)	–

The average exterior diameter of the intestine of the five giant lampreys was 0.56 mm, compared with an average diameter of 1.56 mm for recently transformed American brook lampreys and 0.36 mm for spawning adults.

The eggs in the ovaries of four of the five giant lampreys were still small in late May and early June. The eggs in the other specimen (OR-1), however, were fully developed (average egg diameter, 0.92 mm). The average diameter of eggs from 20 spawning American brook lampreys from Furnace Creek, a tributary of Lake Superior, was 0.90 mm, and Vladykov (1951) reported an average egg diameter of 0.89 mm for American brook lampreys collected during the spawning season in Quebec.

Likelihood of parasitism by American brook lampreys — The giant American brook lampreys either reached their large size as filter-feeding larvae or metamorphosed at a length similar to that of normal brook lampreys and grew to their large size after metamorphosis. The absence of any ammocetes approaching the size of the giant specimens suggests that the giant lampreys did not reach their large size in the larval stage.

Although there is no direct evidence, three factors suggest that the giant specimens may have fed parasitically after metamorphosis: morphological and histological adaptations of the species for parasitic life, the large size, and the absence of extremely large specimens in our samples

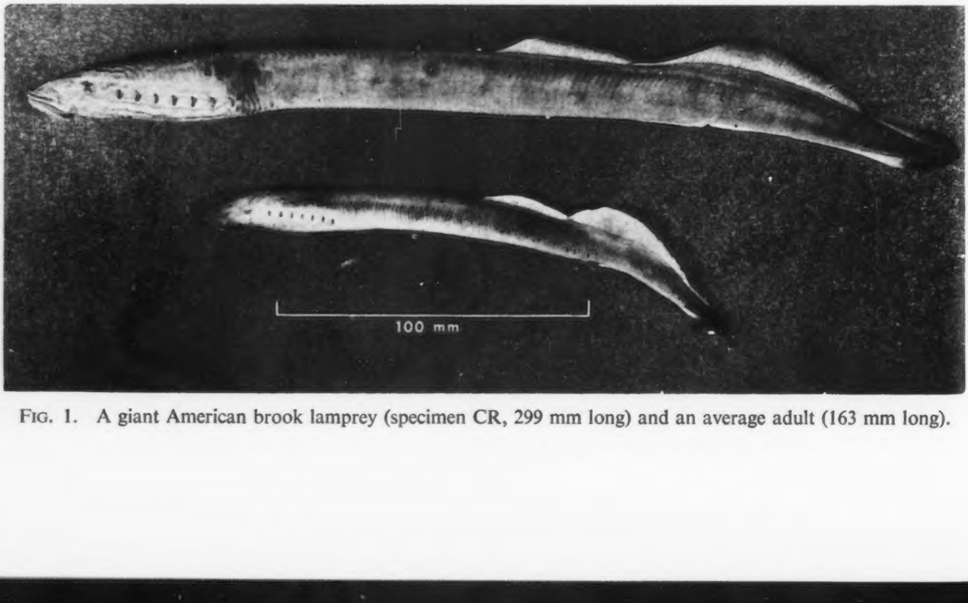

FIG. 1. A giant American brook lamprey (specimen CR, 299 mm long) and an average adult (163 mm long).

TABLE 3. Measurements (mm) of the various characters and numbers of myomeres for five giant American brook lampreys from the upper Great Lakes. (Measurements expressed as percentages of total length in parentheses.)

Measurement or count	Specimen[a]					
	OR-1	OR-2	CR	CLR-1	CLR-2	Avg
Eye diam	4(1.6)	5(1.8)	5(1.7)	4(1.4)	4(1.4)	4(1.4)
Oral disc diam	7(2.8)	8(2.9)	13(4.7)	8(2.9)	10(3.5)	9(3.3)
Length over gill opening	32(12.8)	30(10.7)	36(12.2)	34(12.2)	31(10.9)	33(11.9)
Snout length	16(6.4)	21(7.5)	20(6.8)	20(7.2)	20(7.0)	19(6.8)
Body depth	22(8.8)	18(6.4)	19(6.5)	20(7.0)	18(6.3)	19(6.8)
Body length	190(76.0)	219(78.2)	226(76.9)	216(77.4)	224(78.6)	215(77.3)
First dorsal fin height	7(2.8)	7(2.5)	8(2.7)	6(2.2)	6(2.1)	7(2.5)
Second dorsal fin height	13(5.2)	13(4.6)	16(5.4)	13(4.7)	14(4.9)	14(5.0)
Caudal fin height	4(1.6)	5(1.8)	5(1.7)	5(1.8)	4(1.4)	5(1.8)
Intestine diam	0.43	0.55	0.55	0.37	0.90	0.56
Egg diam	0.92	0.18	0.25	0.26	0.23	0.42
No. myomeres	73	73	72	71	70	72
Total length	260	295	299	286	290	286

[a]OR = Ocqueoc River, CR = Cedar River, and CLR = Carp Lake River.

of over 1 million ammocetes. (The three upstream migrants (OR-1, OR-2, and CR) could have fed in the Great Lakes; the two specimens (CLR-1 and CLR-2) that were taken while migrating downstream could have fed in Carp Lake, which is upstream from the inclined-plane trap in which they were caught.) The American brook lamprey, unlike most other nonparasitic lampreys, has the structures required for parasitic life, including a large sucking disc, sharp teeth, an open esophagus, and buccal glands with anticoagulating secretions (Gage 1928; Baxter 1956). In addition, the alimentary tract histologically resembles that of the parasitic silver and sea lampreys. The absorptive surface of the alimentary canal is increased three to four times by the development of 20–40 longitudinal mucosal folds during metamorphosis (Battle and Hayashida 1965). From 40 to 85 mucosal folds are present in the silver and sea lampreys, but none are present in the nonparasitic northern brook lamprey, *I. fossor* Reighard and Cummins (Battle and Hayashida 1965).

The possibility of parasitism is not ruled out by the small diameter of the intestines (Table 3) or the absence of ingested blood. Spawning migrants of the various species of parasitic lampreys normally cease feeding and the alimentary tract begins to atrophy at the start of the spawning migration. Applegate (1950) noted, for example, that the average diameter of the intestine of the sea lamprey shrank from about 7 mm in feeding adults to less than 2 mm in migrant adults, and Lanzing (1959) reported that in the parasitic species *L. fluviatilus* (L.) Gray, intestine diameters averaged 7–8 mm in early migrants but only about 1 mm in lampreys in spawning condition.

Because American brook lampreys are typically nonparasitic, we can only speculate on the manner of occurrence of giant or parasitic forms. They might have resulted from a genetic mutation, a hormone imbalance, or hybridization, or they may represent the parasitic member in the paired-species concept of Zanandrea (1961).

Hubbs and Trautman (1937) used the term "derivative species" and Zanandrea (1959, 1961) the term "paired species" to describe the morphologically similar parasitic and nonparasitic species in the genera *Ichthyomyzon*, *Lampetra*, and *Eudontomyzon*. Zanandrea (1961) listed *L. lamottei* as one of three nonparasitic species without a corresponding parasitic member. He was undecided as to whether "the ancestor of these species disappeared during the glaciations, or if these nonparasitic lampreys differentiated from other parasitic lampreys, still extant, of the same genus *Lampetra*."

Perhaps the American brook lamprey at one time existed in two forms, the parasitic and the nonparasitic, and the five giant specimens were genetic reversions to an ancestral type.

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A Range Extension for the Squid *Abraliopsis* (*Watasenia*) *felis*

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Fisheries Research Board of Canada
Arctic Biological Station, Ste. Anne de Bellevue, Que.

MacAskie, I. B. 1971. A range extension for the squid *Abraliopsis* (*Watasenia*) *felis*. J. Fish. Res. Bd. Canada 28: 620–621.

Four complete specimens of *Abraliopsis* (*Watasenia*) *felis* McGowan and Okutani, 1968, were collected by net in July 1968 at 48°06′N, 126°10′W. Dorsal mantle lengths of three of these, measured after preservation in 10% formalin, were 19, 23, and 30 mm. Previously reported as found near 43°00′ (McGowan and Okutani 1968), the range of this species is therefore extended 300 miles northwards.

Received December 18, 1970

Under the terms of the Interim Convention on Conservation of North Pacific Fur Seals, Canadian research vessels have collected seal specimens in the area west of Vancouver Island on a course between Amphitrite Point and the Cobb Seamount (Fig. 1).

The stomach contents of all seals taken were examined. Many were found to contain beaks of various species of squid, among which were those of *Abraliopsis* sp. The identification of this genus was based on beaks (U.S. 65-110 CHF) kindly supplied by C. Fiscus of the Marine Mammal Biological Laboratory, Seattle, Washington.

Measurements of the angles CBA, BAD, and ADB on lower beaks (see Fig. 2) provided adequate data for positive identification of most specimens (100% discrimination of *Abraliopsis* beaks from beaks of seven other species using a program for discriminant function analysis developed by Pienaar and Thomson (MS 1969)).

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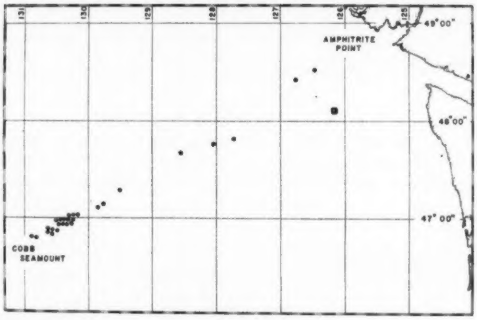

FIG. 1. Location of beaks of *Abraliopsis* (*Watasenia*) *felis* found in seal stomachs (solid circles), and four whole specimens collected by net (solid square).

It is likely that the ingested squid were *Abraliopsis* (*Watasenia*) *felis* as described by McGowan and Okutani (1968). The range of this species is therefore extended further north to 48°32′N, 126°30′W, and west to 46°45′N, 130°49′W.

A more detailed analysis of beaks of these other species, including *Onychoteuthis banksii*, *Gonatus magister*, *Gonatus fabricii*, *Loligo opalescens*, and *Rossia pacifica* will be presented in a later paper.

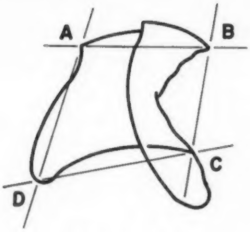

FIG. 2. Lateral view of a lower beak showing angles CBA, BAD, and ADC.

Acknowledgments — The assistance of J. A. Thomson in setting up and running the computer program is gratefully acknowledged.

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Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The number following each title refers to the FRB establishment listed below.

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(4) Fisheries Research Board of Canada
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(7) Fisheries Research Board of Canada
Halifax Laboratory
P.O. Box 429
Halifax, Nova Scotia

(8) Fisheries Research Board of Canada
Biological Station
St. John's, Newfoundland

1489. Freeman, H. C., and D. R. Idler. 1971. Binding affinities of blood proteins for sex hormones and corticosteroids in fish. Steroids 17: 233–250. (7)

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1495. Messieh, S. N. 1970. Immature herring populations in the Bay of Fundy. ICNAF Res. Bull. 7: 59–66. (5)

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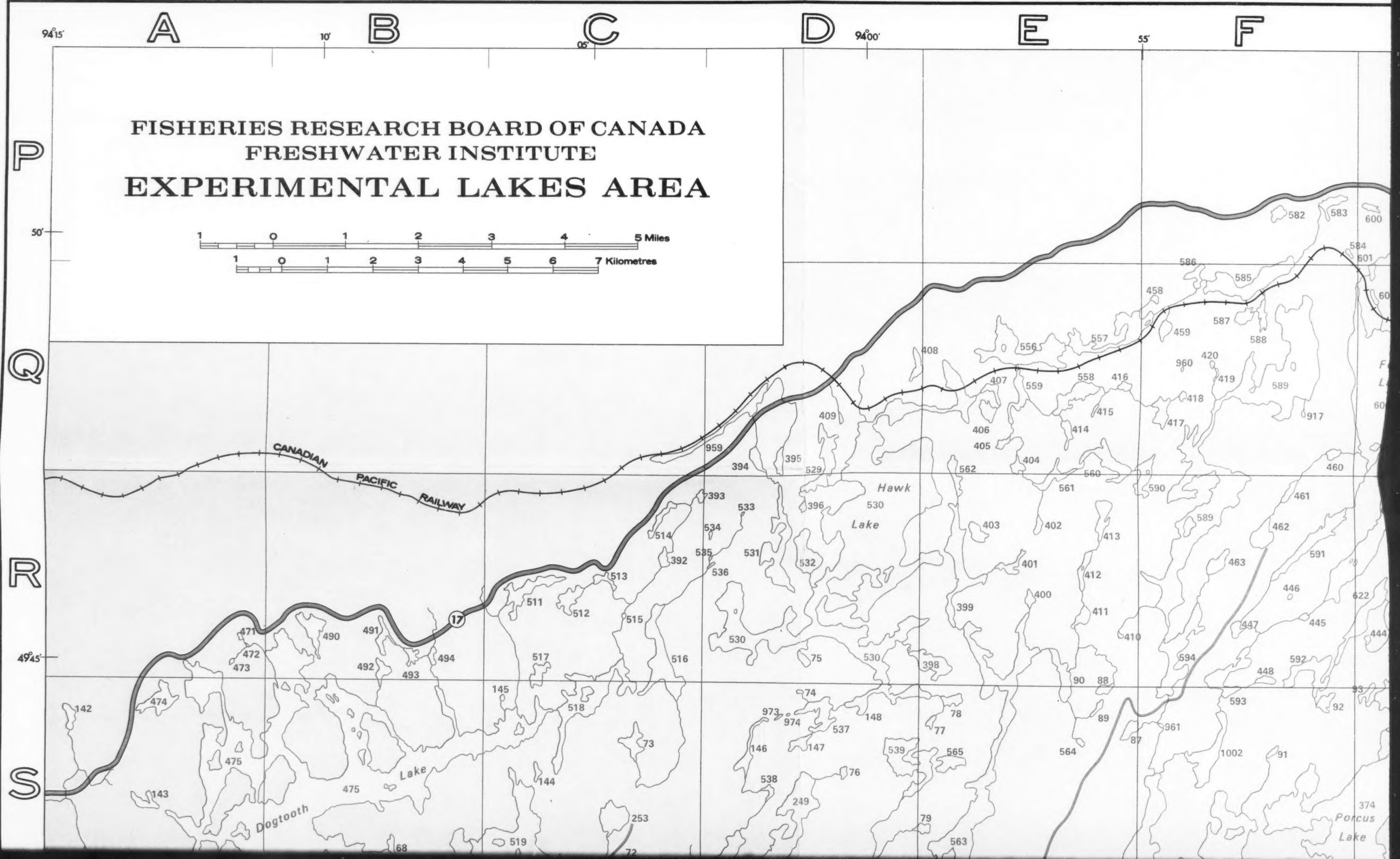

FISHERIES RESEARCH BOARD OF CANADA
FRESHWATER INSTITUTE
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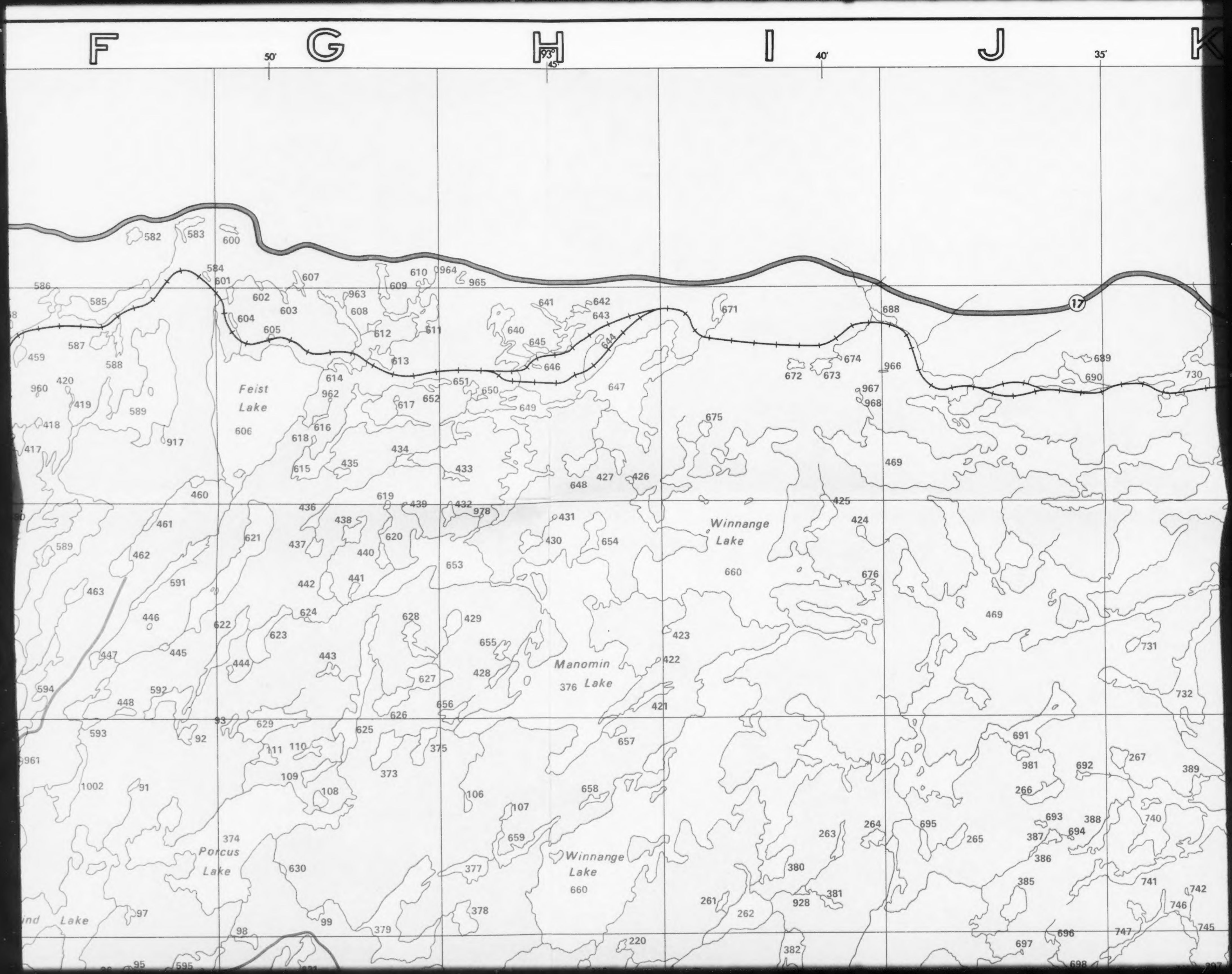
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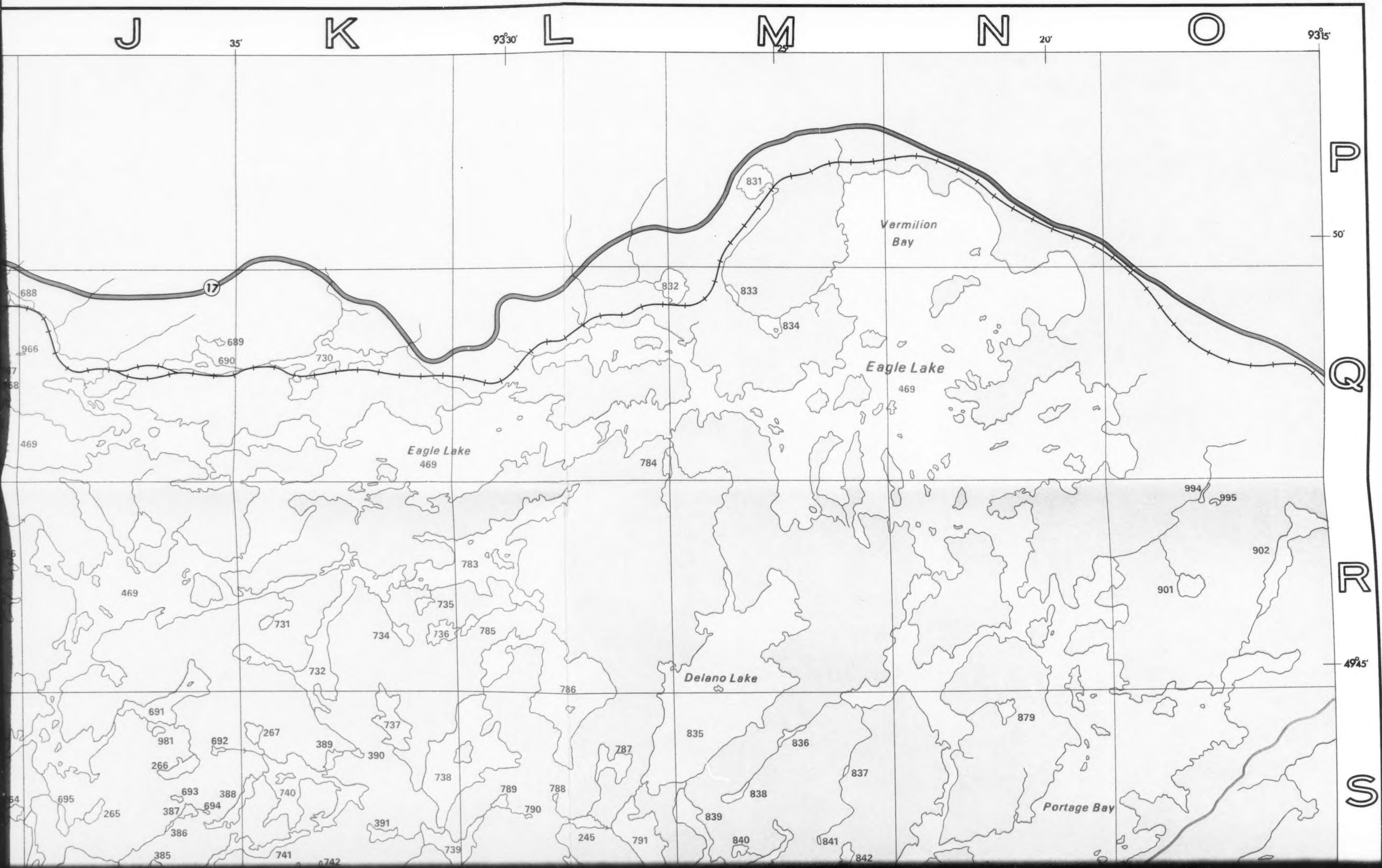
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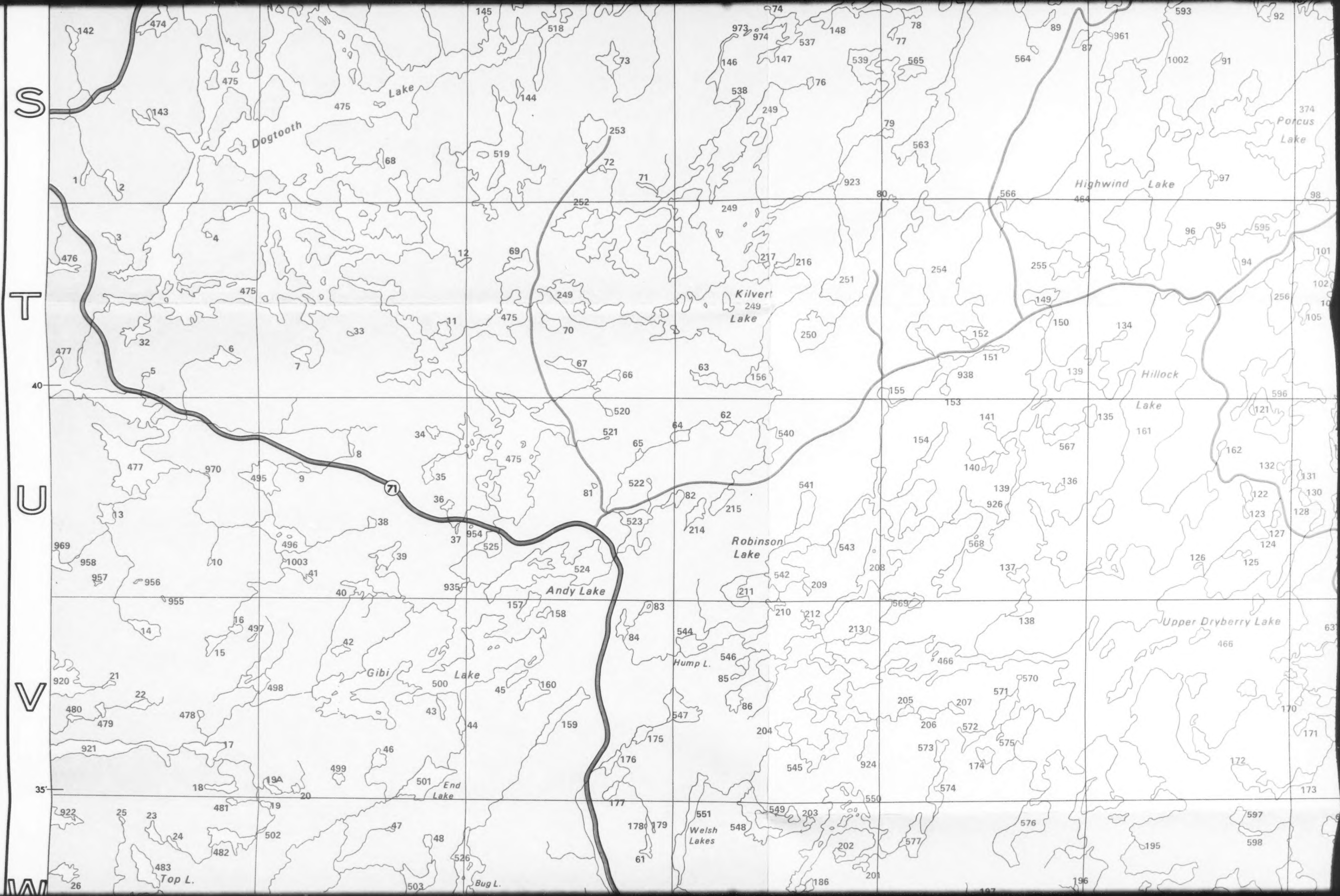
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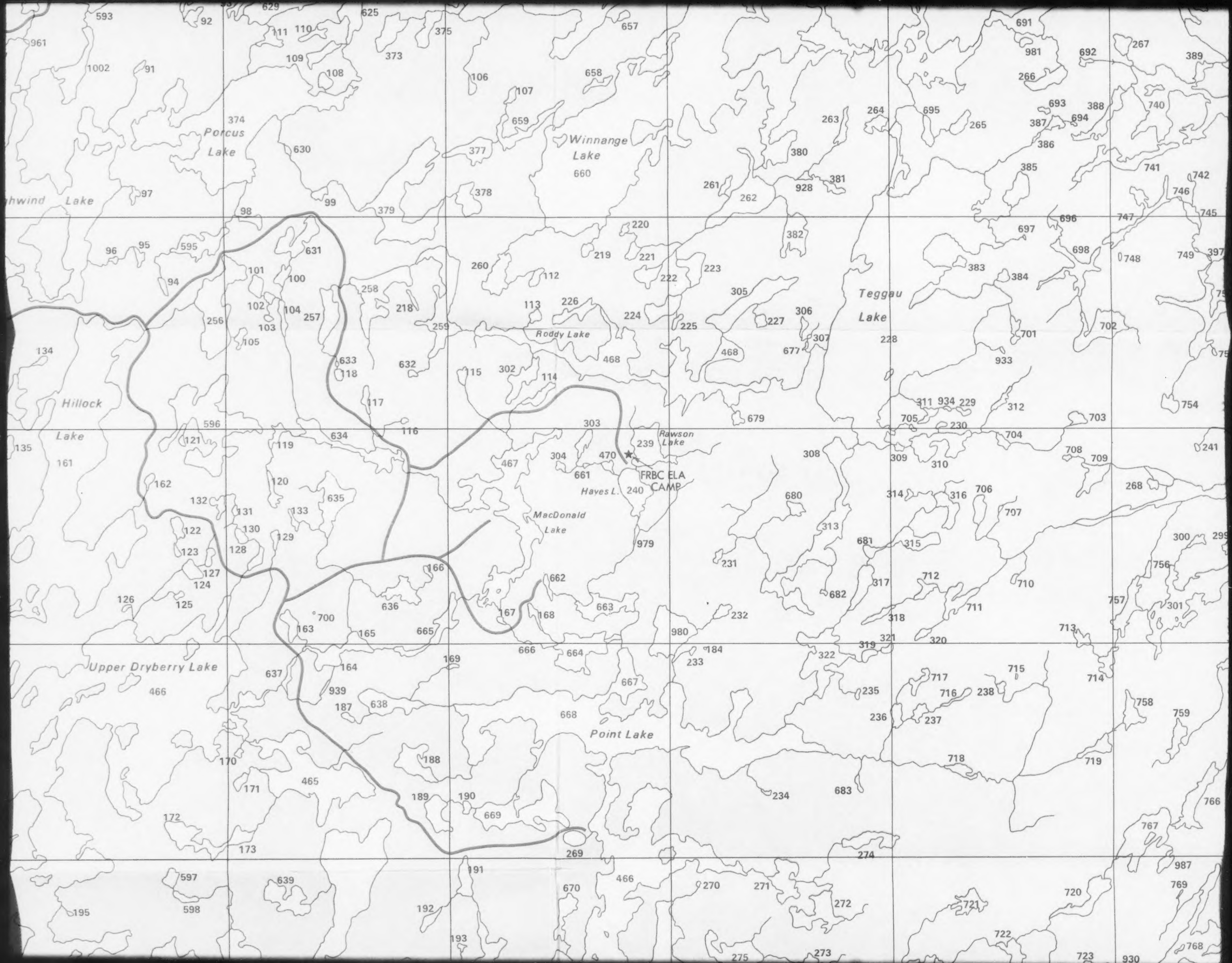
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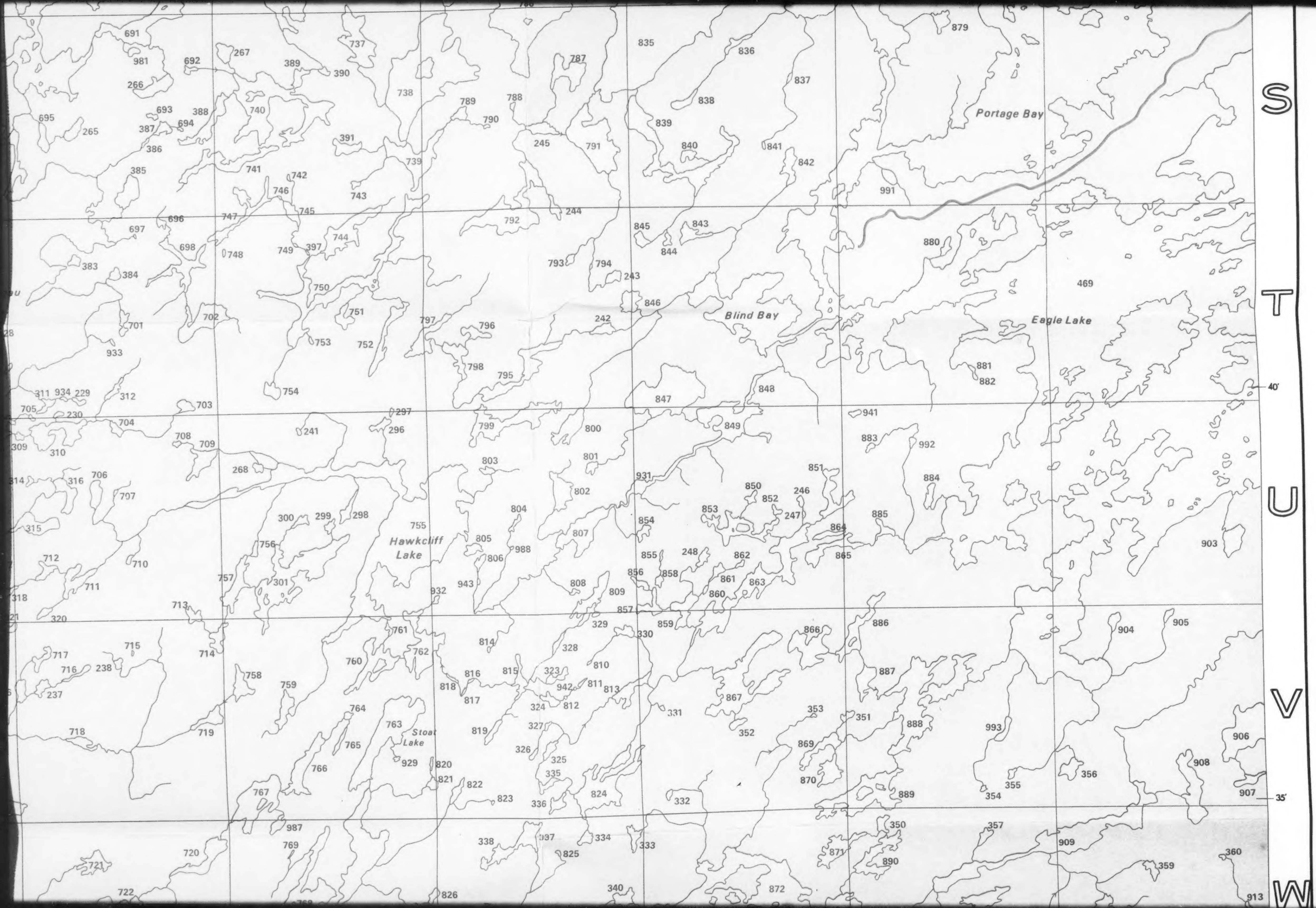

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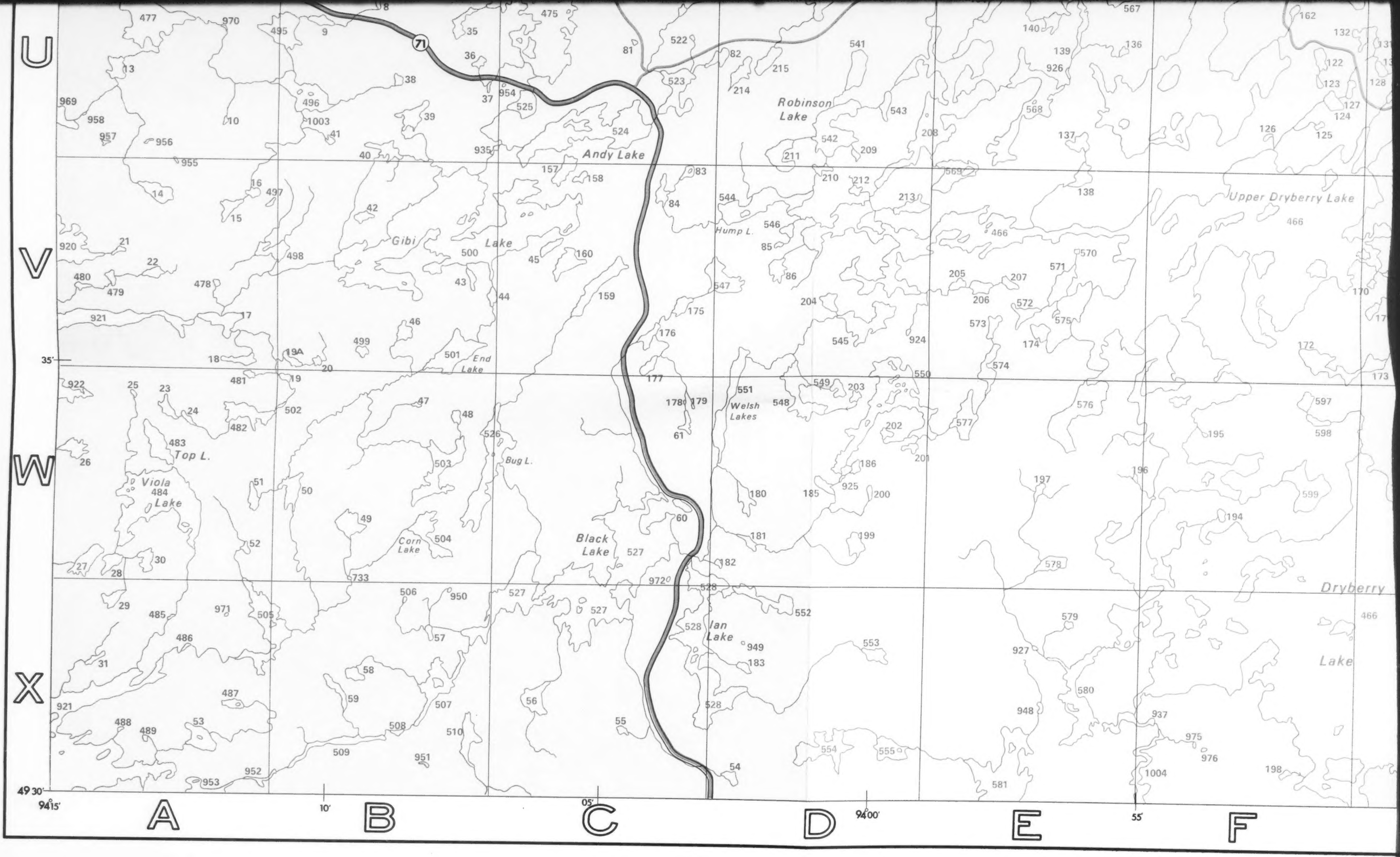
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71
Andy Lake
Robinson Lake
Gibi Lake
Hump L.
Upper Dryberry Lake
End Lake
Welsh Lakes
Top L.
Viola Lake
Bug L.
Corn Lake
Black Lake
Ian Lake
Dryberry Lake
35'
49 30'
94 15'
10'
05'
94 00'
55'
A
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C
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E
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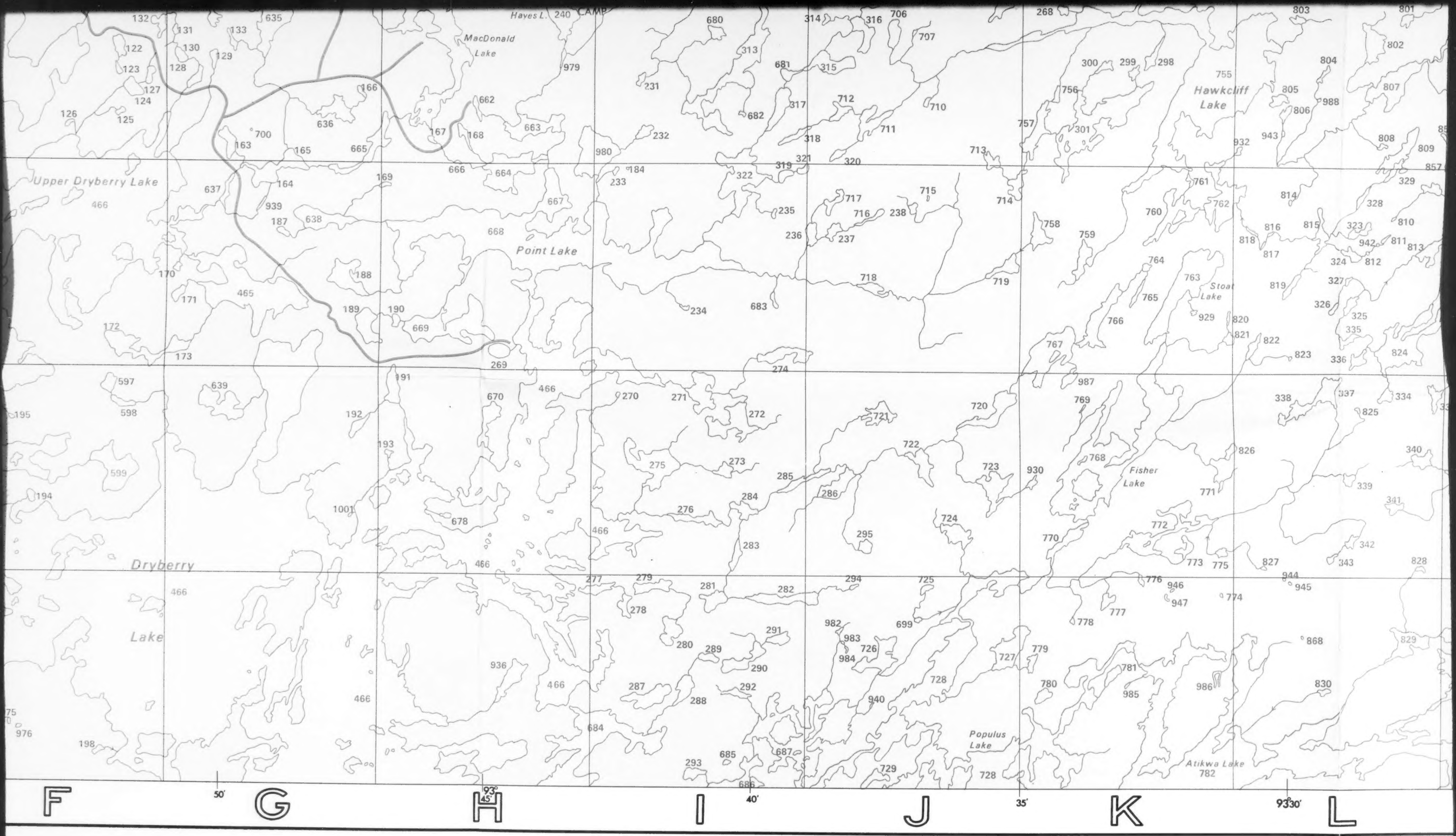
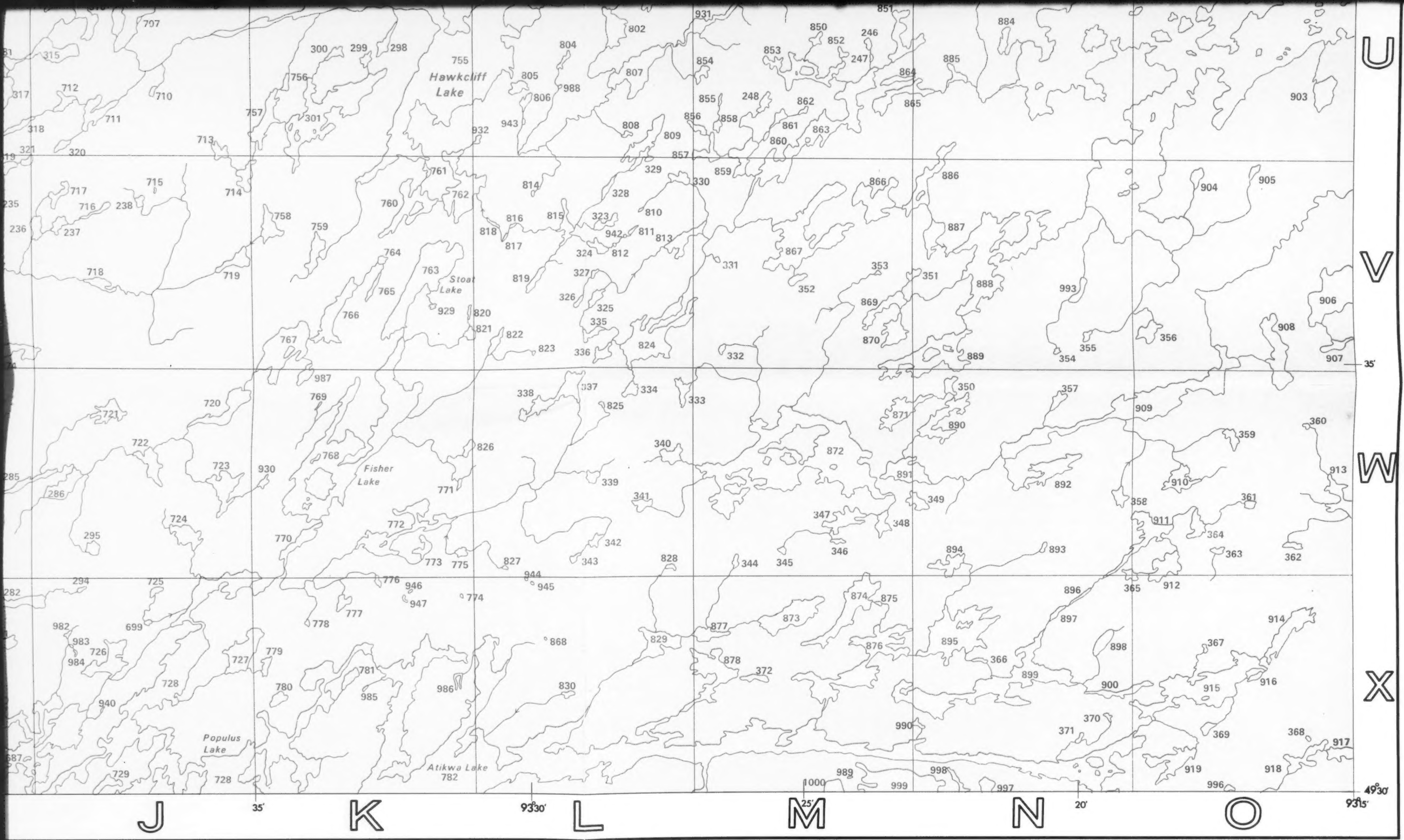

Hayes L.
CAMP
MacDonald Lake
Upper Dryberry Lake
Point Lake
Hawkcliff Lake
Stoat Lake
Fisher Lake
Dryberry Lake
Populus Lake
Atikwa Lake
F
50'
G
93° 45'
H
40'
I
J
35'
K
93°30'
L



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35'
93°30'
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93°15'
49°30'
35'
Hawkcliff Lake
Stoat Lake
Fisher Lake
Populus Lake
Atikwa Lake
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